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ULF von EULER 75 YEARS

On his 75th birthday on February 7th Ulf von Euler may look back on an exceptionally successful scientific carrier. During half a century he and his collaborators have delivered a steady stream of scientific publications which early secured him a leading position in international physiology. Certainly he had some advantages. He grew up in a scientific milieu—his father Hans von Euler became Nobel laureate in chemistry—he had excellent teachers in science—Corneille Heymans in Gent, Henry Dale in London, Goran Liljestrand in Stockholm—and a stimulating working place in Stockholm with Goran Liljestrand and Yngve Zotterman as highly qualified collaborators. But the key to Ulf von Euler's success in science lay in his personal qualities. With the intuitive talents of the born scientist he selected essential problems and tackled them with consistency and intensity. In this way he has made a series of now famous discoveries. Especially worth mentioning are his early observations—already in the 30-ties—on substance P and prostaglandin, contributions which showed to be of paramount importance for later developments. The top of his scientific production was reached by the discovery of noradrenaline as the chemical mediator at postganglionic sympathetic nerve terminals, an achievement which rendered him the Nobel Prize in physiology or medicine in 1970.

Ulf von Euler is since 1957 chief editor of Acta Physiologica Scandinavica. Under his eminent leadership the journal has flourished and its international reputation has become well established.

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Excess cumulative blood flow and repayment during reactive hyperemia in human cutaneous tissue

HANNES K KRISTENSEN and OLE HENRIKSEN

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KRISTENSEN J K. & HENRIKSEN O Excess cumulative blood flow and repayment during reactive hyperemia in human cutaneous tissue Acta Physiol Scand 1980 108 1-6. Received 20 Oct. 1978. ISSN 0001-6772. Department of Dermatology University of Copenhagen Rigshospital, and Medical Department II City Hospital Copenhagen, Denmark.

The influence of duration of vascular occlusion upon the reactive hyperemic response in human cutaneous tissue was studied in 6 subjects. Blood flow in cutaneous tissue was measured dorsally on the distal phalanx of the second finger by the local ^{133}Xenon washout technique. Post-occlusive blood flow calculated from the steepest part of the ^{133}Xenon washout curve just after release of vascular occlusion, reached a maximum value when duration of vascular occlusion was 1 min However excess cumulative blood flow i e the integrated blood flow during reactive hyperemia minus integrated pre-ischemic blood flow for period corresponding to the duration of the reactive hyperemic response increased with increasing duration of vascular occlusion from 3 to 24 min Fractional repayment, excess cumulative blood flow divided by pre-ischemic blood flow times duration of vascular occlusion, was not correlated significantly to duration of vascular occlusion. However there was significant inverse correlation between fractional repayment and pre-occlusive blood flow indicating that, besides metabolic factors, pre-ischemic blood flow in cutaneous tissue is influenced by other factors, such as heat regulation

Key words Reactive hyperemia, cutaneous tissue, normal fingers

The phenomenon of reactive hyperemia in response to vascular occlusion has been studied in many different tissues and organs, arm and leg segments Shepherd 1950 1964 Patterson 1956) hand (Krog et al 1960) finger (Patel & Burton 1956) skeletal muscle (Montgomery et al 1934 Hilton 1953 Lindbjerg 1966) myocardium (Coffman & Gregg 1960 1961), liver (Hanson & Johnson 1966) kidney Honda et al 1968), and adipose tissue (Nielsen & Sejrsen 197? Henriksen et al 1976)

Common findings are that blood flow increases following vascular occlusion and that excess cumulative blood flow i e the integrated blood flow during the period of reactive hyperemia minus the integrated pre-ischemic blood flow for period of the same duration increases with duration of vascular occlusion

No information on the hyperemic response to vascular occlusion in cutaneous tissue has so far been provided

In fingers, the absolute amount and duration of extra blood flow increased with the period of occlusion The blood flow debt repaid showed a large variation but amounted in average only to 50% (Patel & Burton 1956) However since blood flow was measured by venous occlusion plethysmography the contribution of post-ischemic hyperemia in tissues other than skin is included and the results may be influenced by changes in blood flow through arterio-venous anastomoses Using the local ^{133}Xe washout technique, it is possible to measure microvascular blood flow in cutaneous tissue The purpose of the present study was to investigate the influence of duration of vascular occlusion and of pre-ischemic blood flow level upon excess cumulative blood flow and repayment in cutaneous tissue

EXPERIMENTAL PROCEDURE

The experiments were carried out on 6 healthy persons aged 34-4_ years. Room temperature was kept constant at 24°C During the experiments, the subjects were seated on

The number of subscribers have more than trebled—they are today ~1 700 and—quite outstanding for a scientific journal—the economy is good in fact good enough to allow an annual contribution to be distributed as travel stipends among young Scandinavian scientists attending Scandinavian physiology meetings For the success of Acta Physiologica Scandinavica we have to thank Ulf von Euler—and his wife and collaborator Dagmar—for their unselfish and untiring editorial work and care for Acta We collaborators and contributors subscribers and readers all congratulate our chief editor on his 75th birthday and wish him still many active years

Börje Uvnäs

Acta Physiol Scand 1980 108, 1–6

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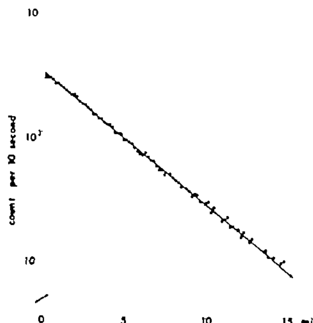


Fig 1 Undisturbed Xenon wa hout curve from the dorsum of the distal phalanx

an adju table chair the legs pendent the arm and hand resting at heart level Cutaneous temperatures on the dorsum of the finger were recorded by thermocouple during the experiments

A kin area of 1 cm diameter just proximal to the nail fold on the dorsum of the second or third left finger was labelled with Xenon using the atraumatic epicutaneou labelling technique (Sejrsen 1971) A NaI scintillation detect r ollimated to rec rd only f om the labelled area and it immediate surroundings was placed 5 cm abo e the labelled area The detector wa connected to a printing gamma pectrometer with a window set around the 0 081 MeV phot peak of Xenon The acti ity wa printed out at 10 interval A miniature blood pressure cuff 20 mm wide onnected to a mercury manometer was pplied around the pro ximal phalanx of the finger Immediately after a labelling period of min the washout f tracer wa followed f r 4 to 5 min Then suddenly the blood pressure cuff w inflated to 300 mmHg nducing ischemi of the finger V scular occlusion wa maintained f r period of 3 6 1 or 4 min After release of the pre sure in the cuff the washout of Xenon w f llowed unt l background acti ty w reached

The count rate ersu time were plotted on semilogarithmic paper after subtraction of background ac ti ity In order t tudy the und sturbed washout of Xenon from the region under study separ te washout curves were btained in all six ubject with inducing scular cclusion (F g 1)

CALCULATIONS

The following l ulat on based n the assumptions of homogeneou perfusion no recircul tion of tracer and flow-limited washout of tracer

A typical reactive hyperemia Xenon washout curve shown in Fig.

1 C ntr l bl od flow (f_{ct}) was calculated from the initial monoexponential portion of the washout curve cording to the formula $f_{ct}=k_{ct}\cdot\lambda\cdot 100$ (ml min 100 g) (Kety 1951) where k denotes the washout rate constant (min) and λ the blood to ti sue partition coefficient (ml/g) A λ value of 0 7 ml/g was used f r cutaneous tissue (Sejrsen 1971)

2 Maximum bl od flow (f_{max}) wa based on the washout rate constant k_{max} calculated from the steepest initial part of the Xenon wa hout curve f llow ng deflation of the cuff $f_{max}=k_{max}\cdot\lambda\cdot 100$ (ml min 100 g)

3 Cumulative bl od fl w V_{cum} (i e the integrated blood flow in the hyperemic period) was calculated for the period t – t (cf Fig) a follows

$$V_{cum} = \int_{t}^{t} f(t)dt \tag{1}$$

where $f(t)$ is the declining perfusion coeff cient in ml min 100 g Under the above mentioned a sumption the externally monit red fractional decrease in activity at time t $-dq(t)/dt$ i given a

$$(-dq(t)/dt)/q(t)=f(t)/\lambda \tag{2}$$

where $q(t)$ i the activity at time t Re arranging equation ( ) and integrating on both ides give

$$\lambda\int_{q(t)}^{q(t)} d\ln q(t) = \int_{t}^{t} f(t)dt = V_{cum} \tag{3}$$

with the solution

$$V_{cum}=\lambda\cdot\ln[q(t)/q(t)] \tag{4}$$

4 Ex al lat bl d fl w V wa determined as c mulative blood flow during the period f react e hyperemia (t t) minu cum lati e post hyperemic blood flow for a period of same duration This gi es

$$V_{ex}=\lambda\cdot\ln[q(t)/q(t)_{ex}] \tag{5}$$

where $q(t)_{ex}$ i obtained by extr polat ng the act ity curve ers s t me from t to t (cf Fig)

5 Fra t onal repa me t R equal V di ided by umulative bl d flow deficit l lated the pr duct of f and the dur tion of scular ccl sion e

$$R=V_{ex}/(\lambda\cdot k\cdot(t-t)) \tag{6}$$

Student t test f r paired or npa red sample were used A limit f ignificance w hosen 0 05

RESULTS

The background-corrected w hout curve obtained in contr l experiment had a m noexponential course over about 1½ decade (Fig 1)

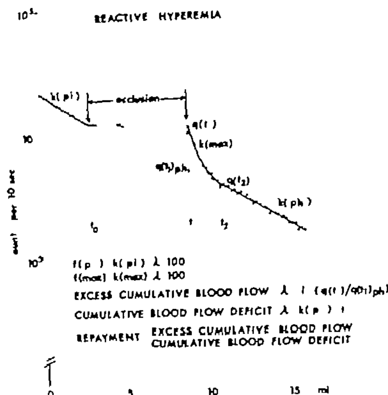


Fig 2 An xample of ^{133}Xenon washout curve pi pre-ischemic, ph posthyperemic

An example of the ^{133}Xenon washout curve is shown in Fig 1 the experiments a washout rate constant, k_{ph} was obtained in a post-hyperemic period which did not differ significantly from that (k) of the pre ischemic period ($P<0.3$) Mean pre-ischemic blood flow f _1 S E was 8 9±4 4 ml min 100 g and post-hyperemic blood flow 8.6 3.0 ml min 100 g (mean of 19 expt)

M mum blood flow increased when the duration of ischemia was prolonged from 3 to 12 min Prolongation of the duration of vascular occlusion to 4 m did not cause further increase in maximum blood flow Fig 3

Duration of react h peremia increased from 0 8 0 1 min to 4 0 9 min, when the period of vascular occlusion was prolonged from 3 to 4 min Fig 4

E es umulat blood flow increased when the duration of ascular occlusion was prolonged from 3 min to 4 min but with smaller increment for the period 1 –4 min than for the period 2 12 min Fig 5

Fractional epaym nt This was on an a erage 82, 73 72 and 69% after 3 6 1 and 4 min of occlusion The values plotted versus pre-ischemic blood flow are shown in Fig 6

DISCUSSION

The described method for calculation of cumulative blood flow is based on the assumption that homogeneous perfusion flow limited washout of tracer and absence of tracer recirculation apply to the area studied by ^{133}Xe washout. This assumption seems alid ince the washout of ^{133}Xe n cutaneous tissue shown in the control xperiment (Fig. 1) followed a monoexponential course to level where remaining activity only amounted to about % of the initial amount of activity This finding can be explained by the observed absence of subcutaneous tissue in the region studied (Hale & Burch 1960)

When furthermor the rest of the hand is shielded off detection of tracer accumulated in fat

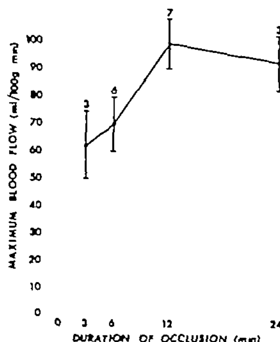


Fig 3 Maximum blood flow determined from the steepest initial part of the ^{133}Xenon washout curve following release of the cuff is plotted against duration of occlusion

ty tissue proximal to the area under study is minimized

The assumptions of a negligible recirculation and a purely blood flow limited washout of tracer seem to be valid for ^{133}Xe (Sejrsen 1971)

Changes in blood volume in the tissue will affect the cutaneous tissue to blood partition coefficient for ^{133}Xe However the influence is less than 3% and can be neglected (Sejrsen 1971) Thus the method seems to be valid under the given conditions

Excess cumulative blood flow was calculated as $\ln[q(t)/q(t)_{pa}]$ (eq 5) where $q(t)_{pa}$ was obtained by retropolating the monoexponential activity curve versus time after the period of reactive hyperemia see Fig 2 An alternative way to calculate excess cumulative blood flow is to draw a line in a semi log plot parallel to the pre-ischemic washout function from t to t_2 starting at $q(t)$ Excess cumulative blood flow would then equal λ $\ln[q(t)_{pa}/q(t)]$ where $q(t)_{pa}$ is the ordinate value of this curve at t_2 This method was not used because the determination of t still depends on retropolation of the final ("post-hyperemic") monoexponential part of the washout curve As already mentioned there was no significant difference between pre-ischemic and post ischemic blood flow

The results obtained are probably not influenced by changes in blood flow through arterio-venous anastomoses for the following reason There is no arterio-venous anastomoses in the area under study (Hale & Burch 1960) Sejrsen (1971) has shown that the washout rate of ^{133}Xe in cutaneous tissue is not affected by flow in the larger veins in subcutaneous tissue

The results obtained are qualitatively similar to those obtained in adipose tissue (Nielsen & Sejrsen 1972) and skeletal muscle (Lindbjerg 1966)

Maximum blood flow increased when duration of ischemia was prolonged from 3 to 12 min Fig 3 Further prolongation did not increase maximum blood flow any further indicating that the arterioles in cutaneous tissue were maximally dilated following 12 min of ischemia However as duration of the hyperemic response increased with increasing duration of ischemia up to 24 min (Fig 4), excess cumulative blood flow still increased when the duration of ischemia was prolonged from 12 to 24 min (Fig 5) The observation that excess cumulative blood flow did show a further increase when duration of ischemia exceeded that necessary to induce total arteriolar relaxation signify that metabolic factors are important in a reactive hyperemia in cutaneous tissue as in other tissue

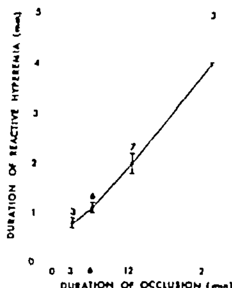


Fig 4 Duration of reactive hyperemia is plotted against duration of occlusion

Decrease in oxygen tension might be important. Fairchild et al (1966) observed that reactive hyperemia persisted in the absence of oxygen. There is considerable evidence that oxygen tension influences arteriolar diameters (Duling & Berne 1970, Hellstrand et al 1977, Hellstrand et al 1977, Duling 1972, Hutchins et al 1974). However increase in carbon dioxide (Kontos & Patterson 1964) and other metabolites might also participate and so might redistribution of ions across the cell membrane and intracellular membranes.

On the other hand, after induction of ischemia the vascular relaxation might initially be partly elicited by the drop in arterial pressure for the following reasons: Prolonging the duration of vascular occlusion from 10 to 30 s did not cause any increase in excess cumulative blood flow in skeletal muscle (Johnson et al. 1976). The hyperemic response was diminished when intravascular pressure was maintained by packing the forearm with blood during vascular occlusion (Patterson 1956). The calculated repayment of more than 200% following vascular occlusion up to 120 sec in the myocardium (Coffman & Gregg 1961) is in agreement with this. However it is not excluded that even after short periods of ischemia repayments exceeding 100% may be elicited by oxygen lack. Myogenic mechanisms seem

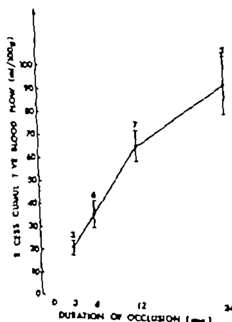


Fig. 5. Excess cumulative blood flow is plotted against duration of occlusion.

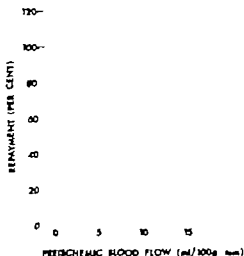


Fig. 6. Repayment is plotted against pre-ischemic blood flow.

quantitatively less important in the present experiments as fractional repayment was maximally 170% (Fig. 6). This might be due to the fact that duration of vascular occlusion was not below 3 min. In skeletal muscle, metabolic factors seem to become important when duration of vascular occlusion exceeds 3 min (Barcroft 1972, Johnson et al. 1976). Furthermore vascular tone in the area under study is highly influenced by sympathetic vasoconstrictor activity which might change the balance between myogenic activity and metabolic factors.

The inverse correlation between repayment and preocclusive blood flow is compatible with the view that normal blood flow in cutaneous tissue is set by factors other than the nutritive demands (e.g. neural impulses governed by temperature regulating centers) or by such factors in addition to those related to the nutritive state of the tissue.

In conclusion, the results of the present study indicate that the reactive hyperemic response in human cutaneous tissue is at least partly due to metabolic factors. However the combined mechanisms underlying the reactive hyperemic response remain poorly understood.

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Effects of rubidium caesium strontium barium and lanthanum on ionic currents in myelinated nerve fibres from *Xenopus laevis*.

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ÅRHEM P Effect of rubidium, caesium strontium, barium and lanthanum on ionic currents in myelinated nerve fibres from *Xenopus laevis* Acta Physiol Scand 1980, 108 7 16. Received 1 March 1979 ISSN 0001-6772. The Nobel Institute for Neurophysiology Karolinska Institutet, Stockholm, Sweden.

A comparative analysis of the effects of externally applied Rb C Sr^{2+} Ba^{2+} and La^{3+} on myelinated fibres under potential clamp conditions was made The ions Cs Ba^{2+} and La^{3+} (belonging to groups 1a, 2a and 3a of period 6 in the periodic system) were found to affect the K system in specific ways Ba^{2+} and La^{3+} reduced the outward K current associated with positive potential steps the effect of La^{3+} being larger than that of Ba^{2+} The effect of Ba^{2+} depended on the K concentration Cs Ba^{2+} and La^{3+} reduced the inward K tail current at repolarisation in solutions with high K concentration C and Ba^{2+} caused rectification of the K current i.e they reduced the inward tail current proportionally more than the outward steady state current The effect of Ba^{2+} could not be described as simple reduction of the permeability constant $\bar{P}$ Rb was found to affect the time course of the K system by increasing the rate constant β_n. Sr^{2+} Ba^{2+} and La^{3+} reduced the permeability constant $\bar{P}_{Na}$ These ions also shifted potential dependent parameters in general along the potential axis. The effect of La^{3+} was larger than that of Sr^{2+} and Ba^{2+} Cs Ba^{2+} and La^{3+} reduced the p-current, Ba^{2+} and La^{3+} the leak conductance

Key words Myelinated nerve ionic currents metal ions, potential clamp analysis

In the present investigation the effects on myelinated nerve fibres of the clawed toad *Xenopus laevis* of alkali metal ions (or group 1*a* ions of the periodic system) Rb and Cs the alkaline earth metal ions (or group ions) Sr^{2+} and Ba^{2+} and the transition element ion (or group 3*a* ion) La were analysed The background was the preliminary finding that Cs Ba^{2+} and La^{3+} affected the steady state P in a rather specific way Ba^{2+} and La markedly decreased outward I_k at relatively low concentration A C Ba and La are the first three elements in period 6 of the periodic system and since it is known that Cs specifically decreases inward I in high [K] test solutions (Dubois & Bergman 1975a, see below) a systematic comparative analysis was performed The analysis also included the effect of the neighbouring ions Rb and Sr^{2+} belonging to period 5 The effect on the ionic currents was studied by the potential clamp technique of Dodge & Frankenhaeuser (1958) and included effects not only on the potassium and sodium systems but also on the unspecific p-current (Frankenhaeuser 1962) as well as on the leak conductance

Earlier investigations have mainly stressed similarities between effects caused by ions within the same chemical group for instance within group 1*a* or group *a* In the present investigation, however I will stress the similarities between effects caused by ions within the same period i e period 6 and to some extent period 5

Among alkali metal ions Li Na K and Rb are known to be highly permeable at least during some phase of the action potential in most excitable membranes. Thus Li and Na have been found to be roughly equally permeant in cephalopod giant axons and amphibian myelinated axons (Baker Hodgkin & Shaw 196, Chandler & Meves 1965 Moore et al. 1966, Hille 1972). I potential clamp

Table 1 *Composition of solutions*

All solutions contained 5 mM Tris-(hydroxymethyl)-amino-methane buffer adjusted to pH 7.

Solution	Concentration (mM) Chloride salt of studied ion	NaCl	KCl	$CaCl_2$
Reference solutions				
Ringer	–	11 0	2 5	2 0
Isotonic KCl	–	–	114 5	0
–	–	57 0	57 0	2 0
Test solutions				
Rb	114.5	–	–	0
	57 0	–	57 0	0
Cs	57 0	–	57 0	0
	10 0	11 0	2 5	0
	10 0	–	114.5	0
Sr^{++} or Ba^{++}	3 4–10.0	11.0	5	0
	3 4–10 0	–	114 5	2 0
La	0 5–1 0	11.0	5	0
	0 5–1 0	–	114.5	0

studies on myelinated fibres from *Rana pipiens* the ratio between the permeability for Li and Na at the peak of the early current has been determined to 0 93 (Hille 1972) In a corresponding potential clamp study the permeability ratio for Rb and K at the steady state level of the late current has been determined to 0 92 (Hille 1973)

On the other hand the alkali metal ion Cs is little permeant in cephalopod giant axons and amphibian myelinated axons Further as mentioned above Cs is known to affect the potassium current in a specific way Externally applied it reduces the inward current in myelinated fibres from *Rana esculenta* (Dubois & Bergman 1975*a*) Internally applied it reduces the outward current in *Loligo* giant axon (Armstrong 1975)

Among alkaline earth metal ions Ca Sr and Ba are known to be very little permeant through the membrane of cephalopod giant axons and amphibian myelinated axons Some other excitable membranes however are highly permeable to these ions during the action potential This is for instance the case for some mammalian and gastropod nerve cell bodies (Reuter 1973 Hagiwara et al 1974)

Further as the alkaline earth metal ions are divalent they share what seems to be a common property of divalent ions in general namely to shift the potential dependent permeability parameters of excitable membranes at relatively low concentrations The classical work here is the potential clamp study of the Ca effects on *Loligo* giant axons (Frankenhaeuser & Hodgkin 1957) in which it was found that Ca shifts the potential dependent permeability parameters of both the sodium and the potassium system This effect of Ca has also been confirmed for amphibian myelinated axons (Frankenhaeuser 1957 Hille 1968 Brismar & Frankenhaeuser 1972) Univalent ions also show such a shift but a smaller one (Mozhayeva & Naumov 1970 Brismar 1973 Hille et al 1975)

Further a similar shift effect on excitable membranes has been found to be caused by other divalent and trivalent ions at relative low concentrations (Vogel 1974 Hille et al 1975 Brismar 1979) The effect has been ascribed to a screening of negative surface charges of the membrane However the situation is complex since there are quantitative differences between the magnitude of the shift caused by different ions in the magnitude of shift of different permeability parameters for one and the same ion and in the magnitude of the shift of the same parameter for different species investigated

Among transition element ions La is known to shift the potential dependent permeability parameters in myelinated fibres from *Xenopus laevis* in accordance with what is mentioned above (Vogel 1974 Brismar 1979)

METHODS

Large myelinated nerve fibres from the sciatic nerve of the clawed toad (*Xenopus laevis*) were used No attempt to distinguish between motor and sensory fibres was made

The effect of the ion in question on the nodal membrane current associated with potential steps were recorded The membrane potential was controlled by the feed back system of Dodge & Frankenhaeuser (1958 1959) The electronic equipment was slightly modified as described earlier (Århem Frankenhaeuser & Moore 1973) The recording cell and the electrode assembly were held at controlled temperature The temperature range in the present experiment was 5–2°C

The experimental procedure was essentially the same as that of Dodge & Frankenhaeuser (1958 1959) The fibre was mounted in a Perspex cell of the design of Dodge & Frankenhaeuser (1958 1959) the internodes on both sides of the node under investigation (N_0) were cut off at different distances from N in different experiments in order to study the effect of possible axoplasmic changes the calomel electrodes were connected to the solution pools by 120 mM KCl bridges the feed-back amplifiers were

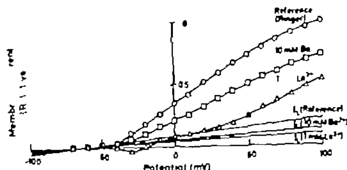


Fig. 1. The effect of Ba^{2+} (10 mM) and La^{3+} (1 mM) on the steady state current-potential relation in test solutions with 2.5 mM K. The net membrane current was measured 6 ms after the onset of the test step. I is leak current. I is obtained by subtracting I_L from the steady state current. Note the inward current at low potential steps for the run with La^{3+}. This is mainly an I_{Na} since the sodium system is not fully inactivated at 6 ms and low test steps. La^{3+} was found to prolong the τ. Holding potential was −90 mV. Axon 19. Temperature 5°C.

...lanced and turned on. The membrane potential was changed in steps, and the associated currents were measured. Measurements were made with test solutions applied to the node under investigation.

The sodium peak permeability associated with a potential step is calculated according to Dodge & Frankenhaeuser (1959) from the peak sodium current, using the constant field equation, the expression for the single ionic current which can be calculated from the Nernst-Planck equation if the electric field inside the membrane is constant (Goldman 1943, Hodgkin & Katz 1949).

The steady state potassium permeability associated with a potential step was calculated correspondingly by the constant field equation from either (1) the steady state current potential relation (I_K–U relation) in solutions of Ringer type (see next section), (2) the $I_K - U_m$ relation in solutions of isotonic KCl type or (3) the instantaneous I at repolarisations in solutions of isotonic KCl type (Frankenhaeuser 1962).

As the currents are given in relative units because of the uncertainty of the calibration factor and consequently the permeabilities also are given in relative units.

Solutions. The composition of reference and test solutions are given in Table 1.

When analysing the experiments and comparing the result some special points have to be taken into account:

(i) $LaCl_3$ is hygroscopic and therefore too high concentration values may be given in the text for some experiments. Further the slow acidification of $LaCl_3$ solution must be taken into account. Thus only fresh solutions were used in the experiments.

(ii) The activity coefficient for divalent and trivalent ions at the ionic strength used in these experiments deviates considerably from 1.0. A theoretical value for the activity coefficient of an ionic species (f) that was used in comparing the efficiency of divalent and trivalent ions is given by the equation

$$\log f = -0.51 z^2 \sqrt{\omega}$$

where ω is the ionic strength of the solution and z the valency of the ion (Morris 1968).

(iii) Many buffer solutions form metal-buffer complexes. The metal-buffer binding constants may be considerable. The only binding constant that may be of importance here is that between Tris buffer and La^{3+} because La^{3+} is used in low concentration relative to the Tris concentration. The value of this was not found. However the binding constants for Tris buffer and Mg^{2+}, Ca^{2+} and Mn^{2+} are known to be negligible (Sober 1968).

Nomenclature. Membrane potential (denoted U_m) is given as inside potential minus outside potential. Consequently outward current is positive. The concepts negative and positive polarisation are used instead of the ambiguous concepts hyperpolarisation and depolarisation.

RESULTS

Effects on the potassium system

Period 6 ions: Cs, Ba^{2+} and La^{3+}. The first step was to confirm that Cs in high concentrations (>10 mM) in test solutions containing high [K] reduced the inward I associated with the repolarisation at the end of a positive potential step, without affecting the outward I associated with the positive potential step.

The next step was to analyse the effects of Ba^{2+} and La^{3+} in more detail. The effect on the steady state current-potential relation is shown in Fig. 1. The main effect of Ba^{2+} on the potassium system in a low [K] test solution was a general decrease of I associated with a positive potential step without any marked effect on the time course of I. The onset of this effect was so rapid that it was impossible in the present experiments to separate the time

Table ? *Effects of Ba² La an l Sr³ on permeability properties*

Effects on P' $\bar{P}_{Na}$ and g_l measured as the ratio between the value in test solution and in reference solution Calcl of P' were made in solutions containing ..5 mM respectively 114.5 mM K (from measurements of sur and stead I_K respectively inward tail I) ΔU is the shift along the potential axi of sodium activation curve (measured a difference between the potential at which peak P_{Na} reaches half its maximum value in Ringer solution and in test s

Tested Ion	Axon	Temp (°C)	P: [K]= 5 mM	P: [K]=114 5 mM, Measurement from: Steady state I	P: [K]=114 5 mM, Measurement from: Tail I	$\bar{P}_{Na}$	ΔU (mV)	g_l: [K] 5 mM	g_l: [K] 114 5 mM
[Ba]=10 mM	5	2	0 67	–	–	0 85	18	1 0	
	17	5	0 77	–	–	0 44	19	1 0	
	18	5	0 63	–	–	0 5	17	1 0	
	19	5	0 74	–	–	0 58	1	0 7	
	30	20	–	1 0	0 43	–	–	–	0 5
	31	20	–	0 96	0.30	–		–	
	3	20	–	0 93	0 45	–	–		
	33	20	–	0 81	0 40	–	–	–	
Mean			0 70	0 9	0.39	0.59	16	0 9	0 5
[La]=1 mM	13	1	0 47	–	–	0 43	17	0 6	
	17	5	0.54	–	–	0 44	19	0 6	
	19	5	0 53	–	–	0.58	16	0 5	
	30	20	–	0 47	0 5	–	–	–	0 5
Mean			0.51	0 47	0 5	0 48	18	0 5	0 5
[Sr]=10 mM	3	–	1 0			0 7?	17	1 0	
	5	–	1 0		–	0 81	18	1 0	
	31	20	–	1 0	0 70	–	–	–	1 0
Mean			1 0	1 0	0 70	0 76	18	1 0	1 0

course of the effect and the time course of the concentration change The effect was further fully reversible So far the effect is consistent with the idea of a decrease of the permeability constant $\bar{P}_K$ (see Frankenhaeuser 1962) The decrease of th maximum value of the steady state P_K for 10 mM Ba² in 4 fibres is given in Table The average decrease was 30%

In order to analyse the effect on inward I (of the Cs effect) and on the potassium activation curve (steady state P $-U_m$ curve) experiments were per formed with high [K] test solutions The motiva tion for this procedure was that the driving force for K in the potential region where P is turned on i larger in a high [K] solution and I is con equently larger

It was found that Ba² like Cs caused an I rectification the inward I_K associated with the re polarisation at the end of a positive step was re duced proportionally more than the steady state outward I during the positive potential tep This is clearly seen in Fig ? and Fig 3 In Fig. ? the effect on the steady state current (ordinate I is obtained by subtracting I_l from the teady state current) as sociated with a potential step (abscissae) while Fig 3 is a corresponding plot of the value of the peak tail inward current associated with the repolarisation (ordinates) from a preceding conditioning step (abscissae) The rectification of the I_K is inconsist ent with the description of the Ba effect as only decrea ing the permeability constant $\bar{P}$ The effect seemed independent of temperature in the range 5–2?°C Further it seemed independent of whether the fibre was ut at the middle of the internodes or at neighbouring node

The effect of 10 mM Ba n the maximum value of P in 4 fibres i hown in Table The P wa calculated from (i) the teady tate I a sociated with positive potential steps or (ii) the peak alue of the inward I tail as ociated with repolarisation The average decrease caused by thi [Ba] in the two ca es wa 8% and 61% respectively

The rectification i evident fr m these calcula tion Another aspect of the Ba effect i further

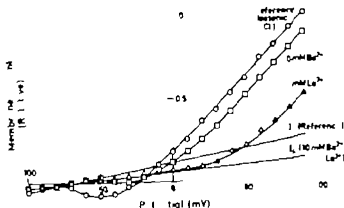


Fig. 2 The effect of Ba (10 mM) and La^{3+} (1 mM) on the steady state current-potential relation in test solutions with 114.5 mM K. The net membrane current was measured at 5 ms after the onset of the test step. The effect on the leak current (I_L) is shown. Holding potential as 100 mV. Axon 30. Temperature 20°C.

ar from Table A comparison between the val s given for the experiments in low respectively gh [K] test solutions shows that Ba^{2+} caused ore pronounced decrease in the steady state I hen external [K] as compared to high. This ght indicate an interaction between K and Ba^{2+} th respect to the effects on the potassium system

expected Ba^{2+} s found to shift the potassium ctivation curve (steady state P – U curve) along he potential axis. This was analysed n experiments th high [K] test solutions in order to obtain an optimal resolution. The shift, caused by Ba corresponded to that of Mg^{2+} and Ca which also are group ions. However the effect of Ba^{2+} as more complex since Ba also caused the rectification of I. The shift of P along the potential axis caused by 10 mM Ba was about 35 mV when estimated from I_K tails in high [K] test solutions.

Ba^{2+} was further found to affect the kinetics of the potassium system. At high potentials (U ≥50 mV) the time constant was increased slightly while at low potentials (U ≤70 mV) as estimated from I tail was decreased. The increase of at high potentials is equivalent to a decrease of the rate constant α_n since at these potentials is proportional to $1/\alpha_n$. Likewise the decrease of τ at low potentials is equivalent to an increase of β_n since at these potentials s proportional to $1/\beta_n$. The effect of Ba was larger on β_n than on α_n.

Concerning the effect of La^{3+} on the potassium system the findings of Vogel (1974) were confirmed that La^{3+} decreased the maximum P. In contrast to the effect of Ba the effect of La^{3+} was only partially reversible. The effect on the steady state current-potential relation is shown in Fig. 1. The decrease of the steady state P caused by 1 mM La^{3+}

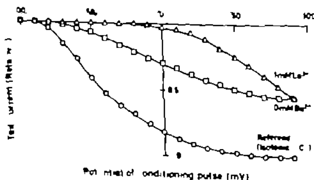


Fig. 3 The effect of Ba^{2+} (10 mM) and La^{3+} (1 mM) on the current tail at repolarisation from potential step to 100 mV in test solutions with 114.5 mM K. Current plotted against the potential of the preceding step. The same axon as in Fig. 2.

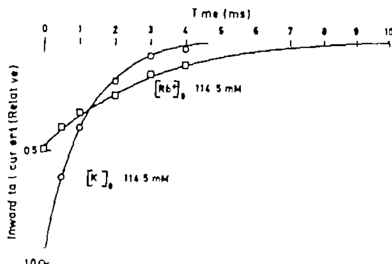


Fig 4 The effect of Rb on τ The tail current at repolarisation to −110 mV from a potential step of 70 mV in 114.5 mM Rb solution and in 114.5 mM K solution Smooth curves are simple exponential curves fitted to the data Time constants used were 1 1 ms and 2.8 ms respectively Axon 4 Temperature ..°C

is presented in Table 2 The average decrease was 49% which is less than the corresponding decrease described by Vogel (1974) The effect on P_K seemed to be independent of the temperature in the range 5–27°C

In order to find out whether or not La³ caused a rectification similar to that caused by Cs and Ba the effect was further analysed in high [K] test solutions Calculations based on the data presented in Fig. ? and Fig 3 indicate an absence of a rectification of the kind caused by Ba La³ caused an equally large percentage decrease of the steady state I and the I_K tail Further measurements indicated that La decreased the permeability constant $\bar{P}_K$ La shifted the potassium activation curve (steady state $P_K - U_m$ curve) along the potential axis similarly as the group ? a ions Mg² Ca² and Ba² An analysis in high [K] test solutions confirmed the results of Vogel (1974) who made measurements in low [K] solutions The shift is clearly seen in Fig 2 and Fig 3 where the effect is shown on the current potential relation in two types of measurements It should be noted however that the steady state I_K was not reached in the experiment with La shown in the figures The magnitude of the shift was large in agreement with the earlier measurements by Vogel (1974)

La was further found to affect the kinetics of the potassium system Large potential steps increased τ independent of whether test solutions with low or with high [K] were used 1 mM La³ increased τ by a factor of 3 or 4 in the poter range 50 to 100 mV which indicates that the ra constant α decreased At low potentials (U ? mV) τ decreased by a factor of to 4 This was studied in experiments with high [K] test solutions (inward I_K tails) This indicates an increase of the rate constant β_n These findings of the effe on τ are consistent with the hypothesis that La shifts the potential dependent parameters along th potential axis

Period 5 ions Rb and Sr² The effects of th period 5 ions Rb and Sr² on the potassium syste were also analysed The well known earlier findi (Hille 1973) was confirmed that Rb can carry th delayed membrane current This was analysed in experiments with an isotonic test solution with high [Rb] and no [K] The outward current associated with large positive potential steps was unchanged while the inward tail current thus here carried by Rb at the end of the potential step decreased about 50% This is consistent with the view that Rb is about half as permeant as K through the potassium transport system This is in contrast to the findings in *Rana pipiens* where the corresponding Rb /K permeability ratio is about 0 9 (Hille 1973)

However when the tail current in a test solution containing 57 mM Rb and 57 mM K was compared with the corresponding current in an isotonic KCl solution the decrease was larger than expected from the measurements in isotonic RbCl solution

Rb was found to affect the kinetics also of the

sium system which was seen in experiments solutions containing high [Rb] and low [K]. time course of the current tail (thus carried ly by Rb) was slow compared to the corre ding current tail in high [K] solution. In the solution with 114.5 mM Rb and no K the time tant τ was about twice as large as in isotonic solution at corresponding steps (Fig. 4). No t by Rb was noted on the time course of the ssium permeability change associated with positive potential steps. Thus Rb affected ly at potentials close to the resting potential or more negative potentials. Since $=1/(\alpha_n+\beta_n)$ α_n is negligibly small at a membrane potential e negative than resting potential it is evident β_n is responsible for the effect of Rb on in potential region

he effect of Sr^{2+} on the potassium system was lysed. As with Mg^{2+} and Ca^{2+} the main effect of was a shift of the potassium activation curve ady state $P - U_m$ curve) along the potential axis shift was for the same reasons as those given were analysed in experiments where the test sol- ion contained high [K]. The magnitude of the ift of the potassium activation curve caused by 10 Sr was smaller than that caused by 10 mM

n contrast to the effect of Ba^{2+} Sr^{2+} caused no or ly a small decrease of the steady state I as- ciated with large positive potential steps. On the er hand in high [K] solutions was found to crease the inward I tail. In one experiment with mM Sr^{2+} a decrease of 30% was observed (Table). The effects of Sr^{2+} were fast and fully reversible

Effects on the sodium system

Earlier potential clamp investigation on amphibian nodes concerning the effects of Sr^{2+} Ba^{2+} and La^{3+} on the Na system (Vogel 1974 Hille et al. 1975 and 1979) were confirmed in the present investigation.

(i) Thus Sr^{2+} Ba^{2+} and La^{3+} decreased the maximum value of peak P_{Na}. A this was seen in experiments where the holding potential was −90 mV or more negative and the inactivation consequently was kept at negligibly low level, it was concluded that this decrease was caused by a de crease of the permeability constant P_{Na}. However some cautiousness in this interpretation is necessary. Thus a change was noted in the shape of the curve relating sodium activation to potential the slope of this curve was less in test solutions containing Sr^{2+} Ba^{2+} or La^{3+} than in the reference Ringer solution. Another complication in the experiments was that the effect of La^{3+} on the sodium system, as in the case of the potassium system, was only partially reversible. The results of the present investigation are consistent with the findings from earlier investigations that the effect of La^{3+} on the peak P_{Na} at large positive steps is larger than that of Sr^{2+} and Ba^{2+}. The decrease of peak P_{Na} was slightly less in the present experiments than that reported by Vogel (1974). It is important here to note the quantitative similarity between the Sr^{2+} and Ba^{2+} effects on the sodium system in contrast to the effects on the potassium system

(ii) Further Sr^{2+} Ba^{2+} and La^{3+} shifted the sodium activation curve along the potential axis in positive direction. The present findings are consistent with earlier findings of the efficiency sequence concerning the magnitude of the shift of the sodium activation curve. Thus

$La^{3+} > Ca^{2+} > Ba^{2+} \approx Sr^{2+}$

The shift caused by La^{3+} was slightly smaller in the present experiments than that reported by Vogel (1974) while the shifts by Sr^{2+} and Ba^{2+} were slightly larger than those reported by Hille et al. (1975)

(iii) Concerning the time dependent parameters a general slow down was observed of the kinetics of the sodium system by Sr^{2+} Ba^{2+} and La^{3+}. This is in accordance with the hypothesis that these ions cause a general shift of the potential dependent parameters. However preliminary calculations showed that the effect of La^{3+} on was larger than expected from the potential shift per se as calculated above from the sodium activation curve

Effect on p-current and leak conductance

Some observations concerning the effects of the ions in question on the non-specific delayed current (the p-current, I) and the leak conductance (g_L) were also made in the present investigation. Thus it was found that the period 6 ions, Cs Ba^{2+} and La^{3+} all systematically decreased I_p. The findings of the effect of Cs were in agreement with the observations of Dubois & Bergman (1975). Caesium affected I_p at lower concentrations (10 mM) than the inward I_K in a high [K] solution. No effect of the period 5 ions Rb and Sr^{2+} on I_p was noted.

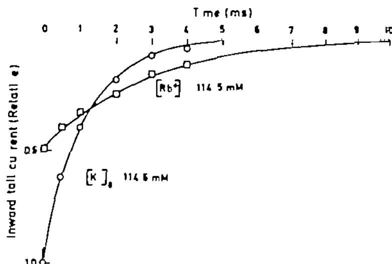


Fig 4 The effect of Rb on The tail current at repolarisation to −110 mV from a potential step of 70 mV in 114.5 mM Rb solution and in 114.5 mM K solution Smooth curves are simple exponential curves fitted to the data. Time constants used were 1.1 ms and 2.8 ms respectively Axon 4 Temperature 22°C

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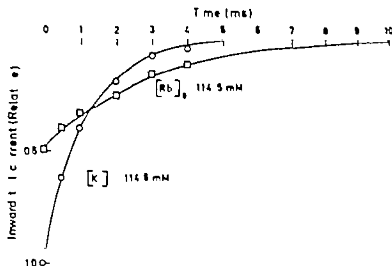


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La³ was further found to affect the kinetics of the potassium system Large potential steps increased τ independent of whether test solutions with low or with high [K] were used 1 mM La³ increased τ by a factor of 3 or 4 in the pote range 50 to 100 mV which indicates that the r constant α decreased At low potentials (U > mV) τ decreased by a factor of ? to 4 This studied in experiments with high [K] test so tions (inward I tails) This indicates an increase the rate constant β_n These findings of the ef on τ are consistent with the hypothesis that La shifts the potential dependent parameters along potential axis

Period 5 ions Rb and Sr² The effects of the period 5 ions Rb and Sr² on the potassium sys were also analysed The well known earlier find (Hille 1973) was confirmed that Rb can carry the delayed membrane current This was analysed in experiments with an isotonic test solution with [Rb] and no [K]. The outward current associated with large positive potential steps was unchanged while the inward tail current thus here carried by Rb at the end of the potential step decreased about 50% This is consistent with the view that Rb is about half as permeant as K through the potassium transport system This is in contra t to the finding in *Rana pipiens* where the corresponding Rb/K permeability ratio i about 0.9 (Hille 1973)

However when the tail current in a test solution containing 57 mM Rb and 57 mM K was compared with the corresponding current in an isotonic KCl solution the decrease was larger than expected from the measurements in isotonic RbCl solution

Rb was found to affect the kinetics also of the

e in low [K] than in high [K]. Dubois & gman (1977) have proposed model consisting pores ith a wider external mouth extending ut halfways nto the membrane The width of external part of the pore was postulated to be ge enough to let C and K move in a single file tern Further they proposed a site with high K nity in the pore the location being about at the e distance from the external as from internal uth of the pore The K site had to be occupied order to keep the pore open The outward I ociated with large potential steps was found to crease under certain conditions when the exter solution contained no K Dubois & Bergman ncluded from their experiments that, within the mes of the presented model Cs c ld replace at the site The Ba nteraction with K found in present in estigation might be interpreted as a ction with the postulated K site Further Rb y also be assumed to react with the K site Test lutions containing no K and 114 5 mM Rb did t decrease the outw rd I

lects on the sodium system

he shift of the sodium acti ation curve by the kaline earth metal ion and by La^{3+} was found to similar to that reported f om *R na pipien* (Hille al 1975) The efficiency sequence was the same hus the findings support the hypothesis of screen g and binding to fixed surface charges in the embrane based on the Gouy-Chapman-Stern ouble layer model (Grahame 1947 Gilbert & hrenstein 1969 B smar 1973 *Hille et al 1975*) he efficiency sequence for *Ran pipiens* and *enopu laevis* differs in one respect from the cor sponding sequence for *Homarus americ nu* Blaustein & Goldman 1968) in the latter case Ba^{2+} auses larger shift than Ca^{2+}

ffects on p-current and leak nductance

he period 6 ions C Ba^{2+} and La^{3+} all decreased e I tail and the p-current as I (I_p) at repolarisa on The effect of C was in agreement with the sult from *Rana escul nt* (Dubois & Bergman 975)

It is known from several investigations that many bstances hich affect I_K also affect I_p. Examples t Na (internally applied Dubois & Bergman 975b) tetraethylammonium ions (TEA both externally and internally applied Koppenhöfer 1967 Koppenhöfer & Vogel 1969) bis-(p-chlorophenyl)-acetic acid (DDA affects the time course Århem & Frankenhaeuser 1974) aniline (Dubois & Bergman 1975*b*) However the situation is complex In preliminary experiments I found that the barbiturate methohex tal increased the outward I associated with large potential steps but decreased I It should also be noted that different amphibian pecies seem to differ with respect to the magnitude of I_p (Armstrong & Hille 1977)

In summary this might seem to support the hypothesis that the I tail is caused by an accumula tion of K in a perinodal space However the hypothesis of an acqeous space bounded by a perinodal barrier (Duboi & Bergman 1975*b*) is inconsistent with several experimental findings Thus the time course of the I_p tail current is voltage dependent and the magnitude of I measured at one potential reaches a limiting maximum value even when the preceding steady state I is increased (Frankenhaeuser 196_). The simple idea that an accumulation of K in a perinodal space is the basis for the I tail must thu be rejected as it was originally rejected by Frankenhaeuser (196_)

Concerning the leak conductance (g_L) the selec ti ity order for the alkali ions found in the present investigation differed in one respect from that reported from fibres in *Rana pipiens* (Hille 1977) Rb was more permeant than C in *Xenopus laevis* fibres It should be pointed out, that no precaution to eliminate a possible contribution from the time and voltage dependent potassium ystem by TEA was taken in the present investigation as was done by Hille in the nvestigation of the *Rana pipiens* fibres In conclusion the results on g_L further stress the fact that the resting potential is not explained by simple electrodiffusion and the constant field assumption (Århem t al 1974 Jack 1976) The increase of g_L in a solution containing 114 5 mM K relative to g_L n Ringer solution was found to be about 20% If electro-d ffusion and the constant field conditions are assumed and further that P_{Na}/P is 0 05 which would be required for resting potential of −70 mV then the predicted increase in g_L would be about 370%

This ork as supported by the S edish Research Council (Project No 14X 345) and Karolinska Institutet fonder

Table 3 *Some physical and chemical properties of the alkali metals the alkaline earth metals and transition element lanthanum*

H_h is standard enthalpy change on hydration at 298 K N 1 electronegativity index Data from Allred (1961) Bockris Reddy (1970) and Harrison (1977)

Group	Ion	Ionic radius (pm)	H (kJ mol)	Mean hydration number (from entr py measurements)	N
Ia	Li	68	−499 1	5	0 98
	Na	98	−389 9	4	0 93
	K	133	−305 4	3	0 82
	Rb	148	−280 7	3	0 8
	Cs	167	− 47 7	–	0 79
a	Be	30	–		1 57
	Mg^{2}	65	−1 891	13	1 31
	Ca	94	−1 561 0	10	1 00
	Sr^{2}	110	−1 413 0	–	0 95
	Ba^{2}	134	−1 272 8	–	0.89
3*a*	La	115	–		1 10

The leak conductance was calculated in the normal way from measurements of the leak current associated with a negative potential step In order to keep the permeability of the potential and time dependent sodium and potassium sy tem at a stable level the holding potential was kept at −90 mV or more negative

Observations were made concerning the selectivity of group I*a* ions on the leak system The leak conductance was measured in experiments where the test solution contained high concentration of the studied ion (see M thods) Some experiments with Li were also included The findings concern ing the leak conductan e were similar to those of Hille (1973) on *Rana pipiens* The selectivity se quence found in the present experiments was K >Rb >Cs >Na >Li This equence for *Xen pus laevis* differs in one aspect from that for *Rana pipien* where Cs Rb

Ba and La were found to decre se the leak conductance (Table 2) The eff ct wa more pr nounced in high [K] solution than in low

DISCUSSION

In the present investigation a comparative analy i was made of the effects on the nodal membrane of the alkali metal ions Rb and Cs (group I*a* peri ods 5 and 6) the alkaline earth metal ions Sr^{2} and Ba^{2} (group 2*a* period 5 and 6) and the transition metal ion La (group 3 *i* period 6) The main finding concerned effects on the potas ium system the ions of period 6 in the periodic system (C Ba² and La) Similar effects but les pronounced wer also obtained by the ions of period 5 (Rb and Sr²) The effects included (*a*) a reduction c steady state P at positive potential steps whic in reased with atomic number and (*b*) a reduc tion of I which decreased with atomic number

Physico-chemical properties that change) tematically with atomic number within period 6 and period 5 are for instance ionic radiu hydration energy electronegativity basic character and tendency for the metal ion to aquire a covalent chara ter when combined with anions (see f r instance Duffy 1974) Value for some of these properties summarized in Table 3 The complexing power c metal ions depends on their harge to radius ra and in the case of transition metal ions on the ligand field stabilisation nergy (cf the Irwing-Williams series of tability) At present it i impossible t explain the described effect on the potassium system by any single phy icochemical property of the types mentioned It hould here be pointed out that while Sr² and Ba diff red markedly in their eff ct on the ste dy tate P at large p tential the differed very little in effe t n peak P

Some findings in the present inve tigation are of pecial relevance f r urrent hyp theses of the molecular mechani m of the p ta sium y tem

Barium decreased the teady t te I calculated from the outward I at positive potential tep

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Effects of physical training on metabolism of connective tissues in young mice

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SUOMINEN H KIISKINEN A. & HEIKKINEN E. Effects of physical training on metabolism of connective tissues in young mice Acta Physiol Scand 1980 108. 17 22. Received 15 March 1979 ISSN 0001-6772 Department of Public Health, University of Jyväskylä, Finland

The effects of physical training on the metabolism of collagen, calcium and glycosaminoglycans in various connective tissues were studied in male NMRI mice. The mice to be trained and their controls were about 3 weeks old (expt. I) and 8 weeks old (expt. II) at the commencement of training The training was performed on a 5° inclined treadmill 5 days a week for 4 weeks in expt. I and for 3 weeks in expt. II The daily exercise time was progressively increased from 20 min in the first week up to 80 min in the third week. The incorporation of ^{3}H-proline to collagen hydroxyproline was increased by training in long bones, skeletal muscle and Achilles tendon, whereas the incorporation of ^{35}S-sulphat into glycosaminoglycans of bones was lower for the trained compared to the control mice. The differences between the two groups in the incorporation of ^{45}calcium were negligible The results indicate that the metabolism of collagen is accelerated by physical training in several connective tissues in young rapidly growing mice, whereas the metabolism of glycosaminoglycans remains unaffected or is even retarded.

Key words Metabolism collagen calcium, glycosaminoglycans, connective tissues, physical training mice

Physical training has been shown to influence both physical and chemical properties of connective tissues (see Booth & Gould 1975 Kiiskinen 1976) The effects of training on the metabolism of different connective tissue components have however been scantly investigated Anderson et al 1971) found an indication of an accelerated mineral and collagen turnover through Sr and ^{3}H-hydroxyproline excretion studies in pigs trained for 2 weeks. Enhanced collagen metabolism of long bones and Achilles tendon was also demonstrated by H proline incorporation measurements in relatively old mice after one month training (Heikkinen & Vuori 1972) In addition some earlier human studies support the idea of accelerated mineral metabolism evoked by physical exercise (Eisenberg & Gordan 1961 Rugan & Briscoe 1964) The authors are not aware of any report describing the effects of training on the metabolism of ground substance glycosaminoglycans

This study attempted to determine the effects of a few week endurance-type training on the metabolism of collagen, calcium and glycosaminoglycans in various connective tissues of young male mice

MATERIAL AND METHODS

Animals and experimental condition

Two separate experiments were carried out. Male NMRI-mice (3 weeks old in expt. I and 8 weeks old in expt II) were randomly assigned to test and control groups The animals to be trained were gradually adapted to running on a treadmill The training was performed on 5 inclined motor-driven treadmill at a speed of 18 m/min, 5 days week for 4 weeks in expt. I and for 3 weeks in expt. II The daily exercise was performed in two sessions, the first in the morning and the second in the afternoon The training time was progressively increased from 2 10 min in the first week up to 2 40 min in the third week. The animals were weighed both at the beginning and at the end of the training period The heart weights served as an indication of training efficiency The trained as well as the control animals were kept in normal laboratory conditions and pelleted mouse diet (Hankkija, Finland) and tap water were given *ad libitum*

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Effects of physical training on metabolism of connective tissues in young mice

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Key words: Metabolism, collagen, calcium, glycosaminoglycans, connective tissues, physical training, mice

Physical training has been shown to influence both physical and chemical properties of connective tissues (see Booth & Gould 1975 Kiiskinen 1976). The effects of training on the metabolism of different connective tissue components have however been scantly investigated Anderson et al 1971) found an indication of an accelerated mineral and collagen turnover through Sr and ³H-hydroxyproline excretion studies in pigs trained for 2 weeks Enhanced collagen metabolism of long bones and Achilles tendons was also demonstrated by H proline incorporation measurements in relatively old mice after one month s training (Heikkinen & Vuori 1972) In addition some earlier human studies support the idea of accelerated mineral metabolism evoked by physical exercise (Elsenberg & Gordan 1961 Ragan & Briscoe 1964) The authors are not aware of any report describing the effects of training on the metabolism of ground substance glycosaminoglycans

This study attempted to determine the effects of a few week endurance type training on the metabolism of collagen calcium and glycosaminoglycans in various connective tissues of young male mice.

MATERIAL AND METHODS

Animals and experimental conditions

Two separate experiments were carried out Male NMRI-mice (3 weeks old in expt I and 8 weeks old in expt II) were randomly assigned to test and control groups. The animals to be trained were gradually adapted to running on treadmill The training was performed on a 5° inclined motor-driven treadmill at speed of 18 m/min 5 days a week for 4 weeks in expt. I and for 3 weeks in expt. II. The daily exercise was performed in two sessions, the first in the morning and the second in the afternoon. The training time was progressively increased from 2 10 min in the first week up to 2 40 min in the third week The animals were weighed both at the beginning and at the end of the training period. The heart weight served as an indication of training efficiency The trained as well as the control animals were kept in normal laboratory conditions and pelleted mouse diet (Hankkija, Finland) and tap water were given *ad libitum*.

Table 1 *Body weights tissue weights and hematocrite values in trained and control mice*
Mean ± S D and number of observations are given

Experiment	Variable	Trained mice		Control mice	
I	Initial body weight g	17 4±1 6	(4)	1 .5± .3	(36)
	Final body weight g	32.4±3 9	(28)	31 7±3 9	(30)
	Dry weight of heart mg	34 1±5 7*	(28)	31 6±4 9	(30)
	Dry weight of humerus mg	38 7±5 0*	(.8)	34 8±4.3	(30)
	Dry weight of femur mg	75 8±12.1	(28)	67 9±9.5	(30)
	Dry weight of m rectus femoris, mg	83.3±1 9*	(28)	77 1±1 1	(30)
	Dry weight of Achilles tendons mg	3 04±0 38	(.8)	88±0 43	(30)
	Hematocrite %	44 0±3 7	(28)	44 3±3 4	(30)
II	Initial body weight g	33 0± 8	(28)	33.±1 4	(36)
	Final body weight g	34 0±2.6	(..)	36 7±3 0	(..)
	Dry weight of heart mg	36 ?±5 2	(2.)	34 7±5 6	(22)
	Hematocrite %	41 8±4..	(..)	44 6±..0	(..)

$P<0\ 10$ $P<0\ 01$ when compared with control group

Metabolism of collagen and calcium (experiment I)
The metabolism of collagen and calcium was examined simultaneously by using a double labelling technique at the end of the training period 60 μCi H-proline (TRA 82 Radiochemical Centre Amersham) and ?0 μC Ca (CES.3 Radiochemical Centre Amersham) were diluted in a physiological saline solution and injected i p into each mouse under ether anesthesia The incorporation was allowed to continue for 4 10 4 and 96 h After each incorporation period 7–8 animals from the two groups were killed by decapitation under anesthesia and blood samples were taken in EDTA tubes for hematocrite (micromethod) and plasma radioactivity measurements Plasma H and Ca-radioact vities were measured by a liquid scintillation counter (LKB Wallac 81000) as described for the bone (see under) Humerus femur m rectu femoris and Achilles tendons were removed and stored at −20°C for subsequent analyses

B Humeru and femur were freed from muscles soft connective t sues and bone marrow and analyzed separately The bones were dried at +90°C for two day weighed (Mettler H20T) and hydrolyzed in 6 M HCl for 16 h at 120°C The hydrolyzates were e aporated to dryness and di solved in 6 ml of distilled water Aliquots were taken for the measurement of both calcium concentration and Ca-radioacti ity Calcium concentration was analyzed by an atomic absorption pectrophotometer (Unicam SP90A) applying the method of Pybus et al (1970) Ca-activity was determined by the liquid scintillation counter by setting the discriminators to the energy level where no H act vity was present For the determinat n of H radioactivity 0.5 ml of the solution was d luted 1 5 ml of distilled water and calcium was precipitated with 0 5 ml of saturated ammonium oxalate at pH 5 (cf Uitto & Lartinen 1968) An aliquot of the supernatant was used for the counting of total H radioacti ity (Prockop & Ebert 1963) The rest of the di solved hydrolyzate (4 ml) was analyzed for H hydroxyproline as described by Juva & Prockop (1966) In all radioactivity measurement a similar sc ntillato (15 g POP and 50 mg POPOP in l toluene together w th 0 6 l methylcellosolve) was used The result were counted as dpm by using an external tandard channel ratio method The counting parameters were calculated u ing quench ng series of Ca and H

Table 2 *C ncentration of hydr xypr line calcium and hex samines (μg/ g dry eight) in v rious tissues f trained and control mi e*
Mean ± S D and number of observation are g en

Experiment	Tissue	Variable	Trained mice		Control mice	
I	Humerus	Hydroxyproline	16 .3 8	(7)	15 9 3 4	(30)
		Calcium	.26±8	(27)	.3±8	(30)
	Femur	Hydroxyproline	17 0 .3 9	(26)	17 5 3	(30)
		Calcium	.5±19	(28)	7 ..	(30)
	M rectus femoris	Hydroxyproline	1 9? 0 7	(.8)	01 0 7	(30)
	Achilles tendon	Hydroxyproline	90 5±9 8	(28)	91 1 8 9	(30)
II	Humeru + femur	Hexosamines	1 34 .0 15	(..)	1 36 0 16	(..)
	Achilles tendon	Hexosamines	53 .0.31	(1)	50 0 1	(1)
	Skin	Hexosamines	1.80 .0 18	(..)	1 83 0 17	( )

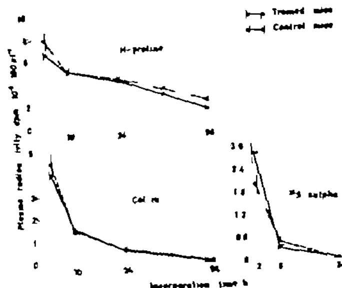


Fig. 1. Plasma radioactivities in trained and control mice. Points represent mean ± S.E. of 5–8 observations. P<0.10 P<0.05 when compared with control group.

proline made in the laboratory immediately before the experiments.

Muscle and tendon. The m. rectus femoris and Achilles tendons of both legs were pooled and analyzed as described for the bones. Only the ³H-proline incorporation results will however be presented.

Metabolism of glycosaminoglycans (experiment II)

The metabolism of glycosaminoglycans was studied by ³⁵S-sulphate incorporation technique at the end of the training period. Isotope ³⁵S-sulphate (SJS...P Radiochemical Centre Amersham) was diluted in physiological saline solution and 2.5 μCi/g body weight was given i.p. under ether anesthesia. After 2, 8 and 24 h incorporation times, the trained and control animals (7–8 mice in each point) were killed by decapitation under anesthesia, and blood samples were taken for hematocrit and plasma radioactivity measurement. Humerus, femur, Achilles tendons and skin were removed and stored at −20°C for further analyses.

Bone. Humerus and femur of the right side were combined and handled together as one sample. After removing the muscles, soft connective tissues and bone marrow the bones were decalcified in 5 ml of 0.05 M EDTA for 3 days. Each bone sample was crushed in mortar and homogenized 5 times for 5 s in 2 ml of ice-cold distilled water by using Sorvall Omnimixer (micro head) kept in an ice bath. The sample was dialyzed against cold running tap water overnight, freeze-dried and weighed. It was then hydrolyzed in 6 M HCl for 16 h at 105°C, and an aliquot was taken for the determination of total ³⁵S-radioactivity. The rest of the hydrolysate was used for the determination of the specific radioactivity of hexosamines. Hexosamines were freed from interfering chromogens by means of Dowex 50 cation exchange resin according to Boas (1953)

after which the concentration was determined as described by Gatt & Berman (1966). The ³⁵S-sulphate radioactivities were measured by the liquid scintillation counter by using similar scintillator and dpm calculation as in expt. I.

Tendon. The Achilles tendons of two animals were pooled and homogenized ten times for 3 s in 2 ml of ice-cold distilled water by using a Potter-Elvehjem-type homogenizer kept in an ice bath. Further analyses were performed as for the bones.

Skin. The skin was shaved, and subcutaneous tissue was scraped off with a blunt knife. A sample of dorsal skin was obtained with a 20 mm punch, cut into small pieces and homogenized and analyzed in the same way as the Achilles tendons.

Statistical method

The results were statistically evaluated by Student's *t*-test (2P) for non-correlating means.

RESULTS

Table 1 presents the body weight, tissue weight and hematocrit values in the trained and control mice. The final body weight of the trained matured mice (expt. III) was significantly lower compared to that of the control mice of similar ages, whereas there were no differences in body weight values between the two younger groups (expt. I). The dry weight of both humerus and femur were significantly higher for the trained versus control mice in expt. I. The heart and skeletal muscle weights also tended to be higher in the trained growing mice. All of the

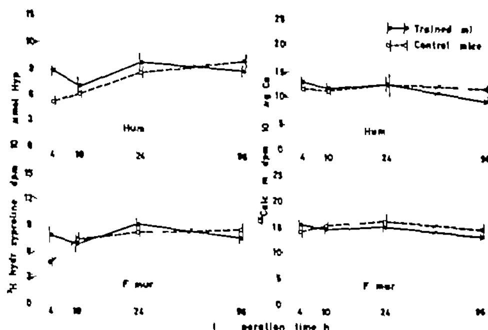


Fig 2 Incorporation of H proline and calcium into connective tissue of bones in trained and control mice in expt I Points represent mean ± S E of 4–8 observations. $P<0.05$ $P<0.01$ when compared with control group

hematocrite values were at the "normal" level although there was a significant difference between the two groups in expt II

As shown by Table 2 there were no significant differences between the trained and control mice in the concentrations of hydroxyproline calcium or hexosamines in any of the studied tissues

The plasma radioactivities after the various incorporation periods appear in Fig 1 Except for some variation in the ³⁵S-sulphate activity the curves were similar for trained and untrained animals

The results of the incorporation of H proline calcium and ³⁵S-sulphate into various connective tissues are shown in Fig 2–4 In every tissue studied the formation of H hydroxyproline was significantly higher for the trained compared to the control mice after 4 h of incorporation An opposite difference between the two groups was observed in the incorporation of ³⁵S-sulphate into bones Although the incorporation of calcium was very similar to that of H proline the differences between the trained and control mice were negligible

DISCUSSION

The enhanced weight development of long bones in the trained growing mice (expt I) is in agreement with the earlier findings of Kiiskinen (1977) as well as Saville & White (1969) The advanced growth of certain tissues did not however appear as differences between the two groups in the gain of body weight As in expt II (Table 1) training rather retards the increase of total body weight with aging, presumably by preventing the accumulation of fat The results further indicate that the hypertrophy of heart and skeletal muscle is more pronounced during the period of rapid growth

The concentrations of hydroxyproline calcium and hexosamines in the tissues studied were not changed by the exercise program used in the present experiments Neither did Tipton et al (1970) observe any changes in the hexosamine concentration of various knee ligaments in trained dogs Some recent findings (Kiiskinen & Heikkinen 1978) however suggest that animal age and the duration of training may have an influence on the response of long bones to exercise

The enhanced incorporation r radioactive proline to collagen hydroxyproline in bones skeletal muscle and Achille tendon of the trained mice particularly during the first hours of the incorporation period suggests that the metaboli m of collagen is accelerated by training This effect is probably not limited to the rapid growth period only because a similar observation was also made on rela-

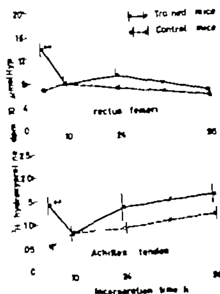


Fig. 3. Incorporation of ³H-proline into connective tissue of skeletal muscle and tendon in trained and control mice in expt. I. Points represent mean ± S.E. of 5–8 observations. $P<0.01$ when compared with control group.

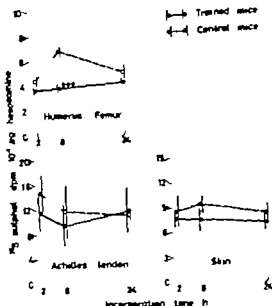


Fig. 4. Incorporation of ³⁵S-sulphate into connective tissue of bones, tendon and skin in trained and control mice in expt. II. Points represent mean ± S.E. of 6–8 observations for bones and skin and that of 3–4 observations for Achilles tendon. *** $P<0.001$ when compared with control group.

tively old mice (Heikkinen & Vuori 1972). Further evidence of a training-induced acceleration of collagen metabolism is given by the increased activity of prolyl hydroxylase in rat skeletal muscle after compensatory hypertrophy (Turto et al. 1974), in mice after treadmill training (Suominen & Heikkinen 1975) and in humans after endurance-type training (Suominen & Heikkinen 1975*b*, Suominen et al. 1977).

The interpretation of the present results on collagen metabolism is disadvantaged by the lack of clear decline in radioactivity during the follow up period and by a somewhat odd "fall" in radioactivity in the trained group after 10 h of incorporation (see Fig. 2, 3), which meant that the actual turnover times could not be deduced. The same difficulties are also true in the estimation of calcium metabolism. In addition, several different pools of calcium exist in long bones (e.g. Laitinen 1976) which makes it difficult to indicate what fraction of bone calcium could possibly be influenced by exercise.

The incorporation of ³⁵S-sulphate particularly into long bones, was somewhat unexpectedly decreased by training suggesting that the response to physical stress is not similar in the different components of connective tissues. Because this observation is of great interest in respect of the age related changes of connective tissues, additional information is needed before definite conclusions can be drawn.

The observed changes in the metabolism of connective tissues evoked by physical training were not quantitatively very dramatic, but it has to be remembered that even small consistent changes may be biologically important in tissues which undergo systematic and progressive life-long alterations (cf. Balazs 1977).

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-ta Physiol Scand 1980 108, 23–29

The effect of divalent and trivalent cations on the sodium permeability of myelinated nerve fibres of *Xenopus laevis*

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BRISMAR, T The effect of divalent and trivalent cations on the sodium permeability of myelinated nerve fibres of *Xenopus laevis* Acta Physiol Scand 1980 108 23–29 Received 30 March 1979 ISSN 0001-6772. Department of Clinical Neurophysiology Huddinge Sjukhus, Huddinge and Nobel Institut for Neurophysiology Karolinska Institutet Stockholm Sweden

The effect of external Ca Mg, Sr Ba and La concentration was studied in potential clamp experiments on single myelinated nerve fibres of the frog (Xenopus laevis). A increase in cationic concentration caused positive shift of the Na permeability (P_{Na}) curve along the potential axis, decrease in max P_{Na} and decrease in the slope of the P_{Na} curve The effectiveness of Mg, Sr and Ba was similar and about 0 6 compared to that of Ca whereas La was about 80 times more effective than Ca

Key words Ca, surface charges, potential clamp myelinated nerve

The concentration of Ca in the external solution affects the excitability of nerve and muscle Frankenhaeuser & Hodgkin (1957) found that an e-fold increase in [Ca] shifted the curve relating peak Na conductance to membrane potential about 9 mV along the potential axis in a direction such that larger positive potential steps now were required to bring about an increase of the Na conductance by a given amount This result was based on a potential clamp investigation on squid axons and the Ca-concentrations used were in the range of 4 4 to 11 mM Frankenhaeuser (1957) estimated on the myelinated nerve fibre the change in the threshold potential for spike initiation to about 6 mV per e-fold change in [Ca] Hille (1968) described a somewhat larger shift 8 7 mV of the Na permeability in the frog node associated with the same change in [Ca]

Other cations than Ca^{2+} influence the relation between Na conductance (or permeability P_{Na}) and membrane potential. In squid axons changes in [Mg] are associated with similar shifts of the sodium conductance curve along the potential axis but the effectiveness of Mg is about 0 6 of that of Ca (Frankenhaeuser & Hodgkin 1957). In the myelinated nerve fibre excitability measurements indicated a somewhat smaller value 0 4 (Frankenhaeuser & Meves 1958) Similar measurements were made on lobster nerve by Blaustein & Goldman (1968) According to these measurements the effectiveness relative to that of Ca would be 0 2 of Mg but larger than 1 0 of Ba. The trivalent cation La has been shown to affect the Na conductance of the lobster giant fibre in a Ca-like fashion but with a 20 times greater potency (Takata et al 1966) In crayfish axons D Arrigo (1973) found that the effectiveness of Ca Mg, Sr and Ba was essentially equal upon threshold membrane potential for spike initiation while it was slightly greater for Co and Ni and much greater (about 60 times) for trivalent cations. On frog fibres Hille Woodhull & Shapiro (1975) showed that the effectiveness of divalent cations on the position of the P_{Na} curve followed the order Ba≈Sr<Mg<Ca≈Mn<Ni<Zn an effectiveness which increased for all ions at higher pH Vogel (1974) compared the effect of Ca and La on the permeability parameters in the myelinated nerve fibre of the frog. He found the effectiveness

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The effect of divalent and trivalent cations on the sodium permeability of myelinated nerve fibres of *Xenopus laevis*

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BRISMAR, T. The effect of divalent and trivalent cations on the sodium permeability of myelinated nerve fibres of Xenopus laevis. Acta Physiol Scand 1980, 108: 23–29. Received 30 March 1979. ISSN 0001-6772. Department of Clinical Neurophysiology, Huddinge Sjukhus, Huddinge and Nobel Institute for Neurophysiology, Karolinska Institutet, Stockholm, Sweden.

The effect of external Ca, Mg, Sr, Ba and La concentration was studied in potential clamp experiments on single myelinated nerve fibres of the frog (Xenopus laevis). An increase in cationic concentration caused a positive shift of the Na permeability (P_{Na}) curve along the potential axis, a decrease in max. P_{Na} and a decrease in the slope of the P_{Na} curve. The effectiveness of Mg, Sr and Ba was similar and about 0.6 compared to that of Ca, whereas La was about 80 times more effective than Ca.

Key words: Ca, surface charges, potential clamp, myelinated nerve

The concentration of Ca in the external solution affects the excitability of nerve and muscle. Frankenhaeuser & Hodgkin (1957) found that an e fold increase in [Ca] shifted the curve relating peak Na conductance to membrane potential about 9 mV along the potential axis in a direction such that larger positive potential steps now were required to bring about an increase of the Na conductance by a given amount. This result was based on a potential clamp investigation on squid axons and the Ca-concentrations used were in the range of 4.4 to 112 mM. Frankenhaeuser (1957) estimated on the myelinated nerve fibre the change in the threshold potential for spike initiation to about 6 mV per e-fold change in [Ca]. Hille (1968) described a somewhat larger shift, 8.7 mV, of the Na permeability in the frog node associated with the same change in [Ca].

Other cations than Ca^{2+} influence the relation between Na conductance (or permeability P_{Na}) and membrane potential. In squid axons changes in [Mg] are associated with similar shifts of the sodium conductance curve along the potential axis but the effectiveness of Mg is about 0.6 of that of Ca (Frankenhaeuser & Hodgkin 1957). In the myelinated nerve fibre excitability measurements indicated a somewhat smaller value, 0.4 (Frankenhaeuser & Meves 1958). Similar measurements were made on lobster nerve by Blaustein & Goldman (1968). According to these measurements the effectiveness relative to that of Ca would be 0.. of Mg but larger than 1.0 of Ba. The trivalent cation La has been shown to affect the Na conductance of the lobster giant fibre in a Ca-like fashion but with a 20 times greater potency (Takata et al. 1966). In crayfish axons D'Arrigo (1973) found that the effectiveness of Ca, Mg, Sr and Ba was essentially equal upon threshold membrane potential for spike initiation while it was slightly greater for Co and Ni and much greater (about 60 times) for trivalent cations. On frog fibres Hille, Woodhull & Shapiro (1975) showed that the effectiveness of divalent cations on the position of the P_{Na} curve followed the order: Ba=Sr<Mg<Ca=Mn<Ni<Zn, an effectiveness which increased for all ions at higher pH. Vogel (1974) compared the effect of Ca and La on the permeability parameters in the myelinated nerve fibre of the frog. He found the effectiveness

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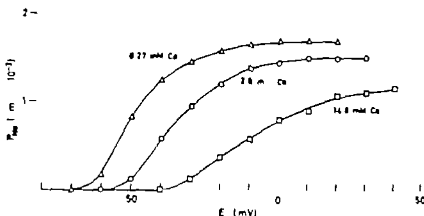


Fig 1 Effect of Ca^{2+} concentration on the relation between the peak sodium permeability and membrane potential change P_{Na} calculated from measurements of peak value of Na current at positive potential steps to various amplitudes indicated on abscissa Membrane potential U = −130 mV between test pulses Temperature 5°C Fibre I

of La to be 55 times that of Ca calculated from the shift of the P_{Na} curve along the potential axis associated with changes in the concentration of these ions

The concentration of univalent cations affects the position of the P_{Na} curve on the potential axis like the concentration of divalent and polyvalent cations (Brismar 1973 Hille et al 1975)

It has been suggested that Ca^{2+} acts on the excitability of nerve through the electrical screening of negative charges which are assumed to be fixed on the external surface of the membrane (McLaughlin Szabo & Eisenman 1971 Brismar 1973 D Arrigo 1973 Vogel 1974 Hille et al 1975) Ca would thereby dependent on its concentration change the electric field in the membrane The hypothesis would in the absence of further complications require that cations of the same valence have essentially the same effectiveness

The present investigation was designed to test this hypothesis Measurements were made of the dependence of the P_{Na} vs potential curve on the concentration of the divalent cations Ca Mg Sr and Ba Similar measurements were further made with solutions containing the trivalent cation La All these ions caused a qualitatively similar displacement of the P_{Na} curve along the potential axis Ca had a larger effect than the other divalent ions while La was much more active than Ca

METHODS

Single myelinated nerve fibres were isolated from the sciatic nerve of the frog (Xenopus laevis) The potential clamp technique developed by Dodge & Frankenhaeuser (1958) was used with some minor modifications (cf Brismar & Frankenhaeuser 1972)

Measurements were made of how the peak initial currents were associated to potential steps of various amplitudes. The peak P_{Na} was calculated from these measurements according to Dodge & Frankenhaeuser (1959) using the constant field equation i e the expression for the single ionic current which can be calculated from the Nernst Planck equation if the electric field inside the membrane is constant (Goldman 1943 Hodgkin & Katz 1949) Such permeability measurements were carried out with the fibre in a number of different concentrations of divalent and polyvalent cations.

Solution The solutions contained 110 0 mM NaCl 5 mM KCl 5 mM Tris buffer (Sigma, pH 7.8 at 5°C) and different concentrations (0 7 0 or 14.8 mM) of $CaCl_2$ (or $MgCl_2$ $BaCl_2$ $SrCl_2$) or one of the following [$LaCl_3$] —91 or 670 nM 5 37 or 770 µM As a reference solution the solution containing 0 mM-$CaCl_2$ was used

Nomenclature Membrane potential (U) is given as inside potential minus outside potential

RESULTS

Potential clamp experiments were performed on single myelinated nerve fibres of the frog (Xenopus laevis) Measurements were made of the effect of test solutions with different concentrations of divalent and trivalent cations on the Na permeability properties of the nodal membrane

The action of [Ca] on the relation between the peak P (calculated from the constant field equation) and the membrane potential (U) is shown in Fig 1 In agreement with the findings in several earlier investigations (see introduction) changes in the [Ca] affected the position of the P curve on the potential axis However the effect was more complex than merely a parallel shift of the permea-

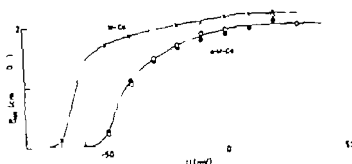


Fig. 2. Effect of zero-Ca solution on peak sodium permeability P_{Na} calculated from measurements of peak value of initial current at positive potential steps to various amplitudes given on abscissa. Membrane potential U −130 mV between test pulses. Solutions applied in the order 2.0 mM-Ca (O), zero Ca ( ) and 0 mM-Ca (●). Temperature 5°C. Fibre I

bility curve along the potential axis. The maximum peak P_{Na} at large positive potential steps was smaller in high [Ca] as compared to low. Furthermore the steepness of the P_{Na} curve decreased with an increase in [Ca]. These effects were reversible. The effect of the [Ca] on the slope of the P_{Na} curve was regular finding. The slope was steeper in low [Ca] also when the P_{Na} curves were scaled in units of the max. peak P_{Na}.

Fig. 2 illustrates an experiment with the node exposed to a solution without divalent cations containing only 112 mM-NaCl, 2.5 mM-KCl and 2.5 mM-Tris buffer. The peak P_{Na} curve was shifted in negative direction and the slope of the curve increased. The limiting value of the peak P_{Na} at large positive potential steps was somewhat larger. The action of a Ca-free solution was not qualitatively different from that of a low [Ca] solution shown in Fig. 1.

Experiments were further carried out with solutions containing various concentrations of one of the following cations: Ca^{2+}, Mg^{2+}, Sr^{2+} and Ba^{2+}. Nerve axons run down more rapidly in low [Ca] solutions than in high (Frankenhaeuser & Hodgkin 1957). Experiments in low [Ca] and in solutions containing other divalent cations than Ca were therefore performed rapidly. Frequent control runs were made in reference solution containing 2.0 mM-Ca in order to give a check on possible irreversible changes in the membrane currents following the exposure to test solutions. Clamp runs showing irreversible changes such as a decreased ratio between specific permeability and leak conductance were discarded. The calculations of effects associated with a test solution were made from comparisons with the mean of the peak P_{Na} values in the reference solution preceding and following the test solution. Errors introduced by small continuous changes in the permeability properties were minimized by this procedure.

Collected data from measurements performed with various concentrations of Ca, Mg, Sr, Ba and La are given in Fig. 3, 4 and 5. Similar to the action of high [Ca] an increase in [Mg], [Sr] and [Ba] was associated with: (i) a shift of the P_{Na} curve in positive direction along the potential axis, (ii) a decrease in the maximum peak P_{Na} at large positive potential steps and (iii) a decrease in the steepness of the peak P_{Na} curve. The action of Mg, Sr and Ba was thus very similar to that of Ca. Fig. 3 represents a plot of the P_{Na} shift (mV) versus concentration (logarithmic scale) of tested cation. The measurements indicated that Ca was more effective than Mg, Sr and Ba. No quantitative difference was observed between Mg, Sr and Ba. The effectiveness of Mg, Sr and Ba relative to that of Ca was estimated to about 0.6. Changes in the cationic concentration caused larger permeability shifts at the high concentration range: about 13 mV per e-fold concentration change at 15 mM and less than 7 mV at 0.27 mM. This is described by the smooth curves which in negative direction asymptotically approach the limiting value estimated in solutions without divalent or trivalent cations. The curves are calculated from the Gouy-Chapman equation relating surface potential to ionic concentration at a certain density of fixed charges at the membrane surface (Grahame 1947, eqn. given in legend of Fig.

Fig 3 Shift of P_{Na} curve vs concentration of different polyvalent cation (C logarithmic scale) The shift (mV) was calculated from the membrane potential at which P_{Na} equals half its maximum in the test solution minus the potential similarly defined in the reference solution containing 2.0 mM-Ca P calculated from the peak value of the Na current The symbols refer to solutions containing either Ca (O) Mg (□) Sr (∇) Ba (Δ) or La (◇) Mean values ± S D Curves are solutions of $\sigma^2 = K\varepsilon_0 RT \Sigma c\,[\exp(-\psi_0 z_i F/RT)-1]$ where σ=charge density (C m) ψ_0= surface potential minus potential of the bulk solution (U) K=dielectric constant of water (=85 90 at 5°C) ε_0=permittivity of free space (=8 85×10 F m) c = th ionic concentration of solution (M m) =valence of th ionic species F=Faraday constant (=96 500 C). R=gas constant (=8 3 J M K) T=absolute temperature (K) This equation is a modification of equation (40) in Grahame (1947) The charge density σ was unknown and assumed to be -33×10 C m which gave a good fit to the measured shift of the P_{Na} curve

3) Earlier measurements of the effect of [Ca] on the P_K curve were satisfactorily fitted by this equation at a charge density of $-5\ 5\times10$ C m (Brismar 1973) The earlier data (Fig. 5 in Brismar 1973) indicated that [Ca] affected the P_{Na} curve more than the P_K curve The present measurements of the P_{Na} curve shift was also better described by the equation at a higher assumed charge density $-33\times10^{-}$ C m (Fig 3)

The concentration of the divalent cations further affected the size of the peak P_{Na} at large positive potential steps The changes in max peak P_{Na} were related to the shift in the P_{Na} curve (Fig 4) The max peak P is expressed in units of max peak I_{Na} in the reference solution containing 2 0 mM Ca An increase in the divalent cationic concentration was clearly associated with a decrease in max peak P_{Na} With regard to this action the effectiveness of the various divalent cations was essentially the same Like the permeability shift described in Fig 3 the change in max peak P was larger in the range of high cationic concentration

Also with regard to the change in the slope of the P_{Na} curve the action of different divalent cations was very similar (Fig 5) The P_{Na} curve was more steep in low cationic concentration the slope of the P_{Na} curve as defined in the legend of Fig 5 was approximately linearly related to the position of the P_{Na} curve on the potential axis (or the P_{Na} curve shift) In solutions without divalent cations P was turned on within a very narrow potential region (Fig 2)

Fig 4 P_{Na} as function of P_{Na} curve shift in different cationic concentrations P_{Na} is max peak P_{Na} given in units of it value in reference solution containing 2 mM-Ca. Max peak P_{Na} calculated by the constant field equation from the peak value of the Na current associated with large positive pulses. Membrane polarized to 170 or −130 mV between test pulses. Straight line drawn through reference point and fitted to the symbol

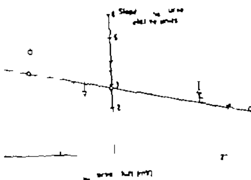


Fig. 5. Relation between slope and shift of P_{Na} curv. Slope measured at the point of the curv. where P_{Na} was half maximum and expressed in arbitrary units defined as incremental change in P_{Na} (of max. P_{Na}) per mV. Straight line fitted to the symbols. The symbols refer to solutions containing either Ca (O), Mg (□), Sr (∇) or Ba (Δ).

The action of the trivalent cation La was investigated. Similar to the divalent cations La acted upon the position of the peak P_{Na} curve on the potential axis (Fig. 6), the max. peak P_{Na} at large positive potential steps, and the slope of the P_{Na} curve. Distinct from the divalent cations La changed the Na permeability properties of the nodal membrane to some extent irreversibly. The decrease in the maximum peak P_{Na} as well as the shift of the permeability curve on the potential axis associated to 0.77 and 2.0 mM-La solutions were not immediately reversible in the reference solution following the exposure to La. The time course of the changes in the permeability properties are plotted in Fig. 7.



Fig. 6. Effect of La on Na permeability. Peak P_{Na} calculated from measurements of peak value of Na current associated with changes in membrane potential to different levels as indicated on abscissa. Membrane polarized to 120 mV between test pulses. Measurements in 2.0 mM-Ca (O), 1 min after beginning of solution change to 0.77 mM-La ( ), and 1 min after beginning of washout in 2.0 mM-Ca (●). Temperature 15°C. Fibre No. 11.

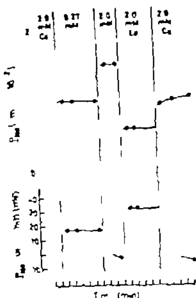


Fig. 7. Time course of La action on max. peak P_{Na} (top) and P_{Na} curv. shift (bottom). Vertical lines mark beginning of solution change which required about 15 s. Each permeability curve was obtained from a family of test pulses applied during about 30 s, indicated on the abscissa is the time at the completion of each pulse family. Same axon as in Fig. 6. Temp. 15°C.

The onset of La action was rapid and complete before the test pulses were applied, i.e. it cannot have required more than 30 s. During washout the permeability curve gradually shifted to the original position on the potential axis while the original amplitude of P_{Na} was incompletely restored. There was obviously a damage to the permeability mechanism associated with the exposure of the fibre to high [La] solutions. This effect may be separate from the reversible action of La on the potential dependence of the P_{Na} curve. Estimates of changes in max. peak P_{Na} in different [La] were therefore ambiguous. The quantitative description in Fig. 3 of the effect of various [La] was made from a comparison with the mean of the peak P_{Na} values in the reference solution preceding and following the test solution. The Figure shows, that a change from 2.0 mM-$CaCl_2$ to 1/80 as much $LaCl_3$ in the external solution would produce no permeability shift. The shift associated with an e-fold change in [La] was about 8 mV in the range 5 μM to 2.0 mM. Below 5 μM the effect of a decrease in [La] was small and approached the limiting value measured in solutions without divalent and trivalent cations.

DISCUSSION

The effects of divalent and trivalent cationic con centration on the Na permeability properties of the nodal membrane have been described From meas urements of peak Na current at various membrane potentials the peak P_{Na} was calculated from the constant field equation (Dodge & Frankenhaeuser 1959) and plotted against the membrane potential The analysis of such curves revealed that an in crease in concentration of all cations investigated (Ca Mg Sr Ba and La) shifted the P_N curve to more positive potentials and decreased the max peak P_{Na} There was further a decrease in the slope of the P_{Na} curve in high concentrations of these ions The effect on the permeability properties of an e fold change in [Ca] was greater in the range of high [Ca] This was also the case for changes in *[Mg] [Sr] [Ba] and [La] It is further known* (see introduction) that the effect of [Ca] depends on the concentration of other salts present in the solu tion For these reasons it is difficult to interpret the quantitative difference in Ca-effects on prepara tions with large difference in the salt concentration of the external solution The obtained results were in agreement with investigations made by Franken haeuser (1957) Frankenhaeuser & Meves (1958) Hille (1968) and Vogel (1974) of different [Ca] in ordinary Ringer's solution on the nodal membrane of frog fibres It is interesting to note that the Na permeability shift along the potential axis measured in Ringer s solution containing about 11? mM NaCl was about 1 5 times greater than the corresponding shift in solutions of 25 0 mM NaCl plus 90 0 mM KCl *(Brismar 1973)*

The relative effectiveness of other divalent cat ions compared to Ca was not dependent upon the concentration range It was for all of them (Mg Sr and Ba) estimated to about 0 6 A similar esti mate was made by Frankenhaeuser & Hodgkin (1957) on the squid giant axon and by Franken haeuser & Meves (1958) on the frog myelinated nerve fibre Hille et al (1975) found the same dif ference between the effectiveness of Mg Sr and Ba compared to Ca in frog fibres but distinguished that Mg was *slightly more effective than* Ba and Sr The lanthanum ion La^3 which was the only trivalent cation tested had essentially the same action as the divalent cations but its effectiveness was con siderably greater La differed from the divalent cations in the respect that solutions containing 0 7 mM La or more had partially irreversible effects on the Na permeability properties It has ea been reported (Takata et al 1966) that the ac of La on the Na permeability properties of t lobster axon gradually increases during sev minutes exposure This was not the case for t La-effect on the nodal membrane

The greater effectiveness of Ca compared other divalent cations belonging to the same gr of elements has some definite implications on t hypothesis of Ca-action on the excitable membr (see introduction) If it is assumed that the [C affects the position of the Na permeability cur on the potential axis because of an interaction b tween Ca and fixed negative charges at the exte nal membrane surface it is necessary to enck some assumptions which account for the differen between cations of the same valence Several i *vestigators (Gilbert & Ehrenstein 1969 Mozhaye* & Naumov 1970) have suggested that Ca could bi to negatively charged group at the surface an thereby neutralize the negative charge The bindi coefficient might differ between for example M and Ca In an investigation of the action of differe divalent cations on the threshold for excitation crayfish axons D Arrigo (1974) found that pH i fluences the effectiveness of the different ions Th effect he explained from the action of pH on t electric field strength of fixed negative charges a the theory of alkaline-earth equilibrium selectiv (references in D Arrigo 1974)

The basic idea in these hypotheses is that positi ions in the external solution interact with negativ charged groups on the external membrane surfa *According to this ions with higher* valence wo have considerably greater effect So far the findi that La is much more effective than the divale cations agrees with these hypotheses This d ference between the effectiveness of La compar to the divalent cations was approximately describ by the model of cationic screening of fixed neg tive charges (density −33×10 C m) at the nod membrane surface This value is considerab higher than earlier calculated to describe the effe of [Ca] on the P properties (−5 5×10 C m Brismar 1973) A similar difference between t effect of Ca and La on the P_{Na} and the P propert led Vogel (1974) to assume that the charge densi was about −23×10 C m at the Na permeabil site and about −8 0×10 C m at the K site

Other effects of changes in divalent and trivale *cationic concentration* such as the change in ma

...n & P_{Na} and in the slope of the P_{Na} curve were ...ssently not explained by the model If however ... effect of a negative surface potential is ac... ...unted for in the constant field equation as was ...done by Frankenhaeuser (1960) in his comparison ... P_{Na} in frog and squid axons, the surface charge ...odel of Ca action will also cover (at least quali-...tively) the effect on max. peak P_{Na}. This model ...edicts changes in I_{Na} rectification which may be ...sted by the analysis of I_{Na} tails at repolarisation ...ter positive pulses. Such an analysis was not ...cluded in the present investigation.

...is work was supported by the Swedish Medical Re... ...arch Council (Project No. 14X-4255) and the Fonda...ns of Karolinska Institutet

SUMMARY

...otential clamp experiments were performed on single myelinated nerve fibres from the frog (Xenopus laevis). The Na permeability (P_{Na}) properties of the nodal membrane were studied in different outside concentrations of ...ne cations Ca, Mg, Sr Ba (divalent) and La (trivalent). At ...n increase in cationic concentration ( ) the P_{Na} curve was ...ifted in positive direction along the potential axis, ( ) ...e max. P_{Na} decreased, and (iii) the slope of the P_{Na} curve ...ecreased The effectiveness of Mg, Sr and Ba was similar ...d about 0 6 compared to that of Ca, whereas La was ...bout 80 times more effective than Ca The hypothesis of ...ionic action through screening of fixed negative ...arges on the nodal membrane surface predicted ap...roximately the difference between La and Ca at an ...umed charge density of 33 10^{-4} C m^{-2}

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Alkali metal cation transport through the human erythrocyte membrane by the anion exchange mechanism

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This work deals with the possibility that cations may be transported through the human red blood cell membrane as negatively charged ion pairs of the type XCO Passive sodium influx into human erythrocytes was increased 4-fold when 150 mM chloride was substituted with bicarbonate in the medium This increased permeability to sodium was completely abolished when the cells were pretreated with specific inhibitor of anion transport (DIDS) Both bicarbonate-induced sodium influx and chloride exchange were linearly related to DIDS-binding Both transport processes were completely inhibited by the binding of 1.1 10^6 *DIDS molecules per cell The presence of bicarbonate and/or pre-*treatment of the cells with DIDS did not change the permeability of the erythrocyte membrane to K Rb and C to any significant extent. The experimental findings support the hypothesis that Li and Na but not K Rb and C form monovalent negatively charged ion pairs with CO which traverse the membrane through the anion exchange system

Key words Anion transport, anionic cation transport bicarbonate carbonate ion pairs cation transport, erythrocytes

Sodium and lithium transport through the red cell membrane is very sensitive to the presence of HCO which increases the membrane permeability to the cations It has recently been demonstrated that the bicarbonate-stimulated Li influx is abolished by inhibition of the specific anion exchange system of the red cell membrane (Funder Tosteson & Wieth 1978) This finding gave support to the notion that the increase of Li flux is due to the formation of negatively charged anion pairs (LiCO) which can be transported by the anion pathway (Wieth 1970). The purpose of the present article is to investigate whether the bicarbonate stimulated Na transport also can be quantitatively related to the function of the anion exchange mechanism In addition it is examined whether the stimulation of alkali metal cation transport with bicarbonate is limited to Li and Na Potassium, rubidium, and cesium ions are not supposed to form ion pairs with carbonate. The ion pair hypothesis therefore implies that bicarbonate will not stimulate passive transport of other alkali metal cations than Na and Li

METHODS

Freshly drawn heparinized human blood was centrifuged and the plasma and buffy coat were discarded Unless otherwise noted the cells were washed 3 times in the medium in which the experiment was performed After the final wash the cells were suspended at hematocrit of 30% and the cell suspension was incubated at 38°C and pH 7.4 Ouabain 10 M was added 5 min before start The technique of incubation, control of pH and P and isolation of cells and media at 0°C has been described by Funder & Wieth (1967). Sodium and potassium were determined by flame photometry and the cell solids were determined by drying the cells to constant weight at 105°C for 24 h. All cellular concentrations were corrected for the trapping of 2.4 (wt/wt) of extracellular medium in the packed cell columns.

Pretreatment of erythrocytes with DIDS and the experimental procedure in the experiments with graded

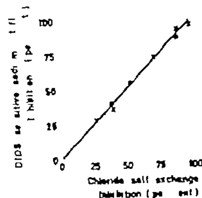


Fig. 1. Comparison of the DIDS-induced inhibition of chloride self-exchange and of the bicarbonate-dependent net flux. Human red cells were preincubated with varying concentrations of DIDS resulting in irreversible binding of between 2.5 10^{6} and 1.2×10^{6} DIDS molecules per cell. After the DIDS treatment one sample of cells was used for the determination of chloride self-exchange. The other sample was washed 3 times with 150 mM NaHCO$_3$ at room temperature, which according to chemical analysis caused complete replacement of cellular chloride with bicarbonate. The cells were then suspended in 150 mM NaHCO$_3$ medium and the Na transport was determined at 38°C and pH 7.4. The results (obtained with cells from two donors (●)) demonstrated that the chloride transport and the bicarbonate-sensitive sodium net flux were equally reduced by DIDS (correlation coefficient (r)=0.995). The bicarbonate-stimulated Na net flux was reduced from 16.4 to 2.5 mmol kg cell solids at 100% inhibition.

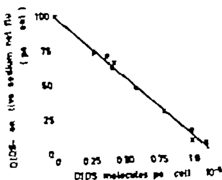


Fig. 2. The relation between the number of DIDS molecules bound per erythrocyte and the degree of inhibition of the DIDS-sensitive bicarbonate-stimulated Na net flux. (Regression line: $y=-89.4x+100.9$, correlation coefficient 0.994.) Complete inhibition of the bicarbonate induced sodium transport is obtained by the binding of 1.13 10^{6} DIDS molecules per cell. The results are obtained with cells from two donors ●.

from zero to 150 mM (substituting Cl with HCO$_3^-$) increased the Na influx from 7 to 25 mmol kg cell solids h. A similar effect is seen on the net flux of Na into ouabain-treated cells. Table 1 shows that this effect of bicarbonate is completely eliminated if the red cells have been treated with the amino-reagent DIDS, which so far has proved to be a specific inhibitor of anion transport in erythrocytes. It is obvious that increase of the bicarbonate concentration from zero to 150 mM has no effect on the influx of Na into DIDS-treated cells.

The quantitative relation between graded inhibition of bicarbonate-stimulated Na net flux and graded inhibition of the anion exchange system is shown in Fig. 1. There was identity between the fractional inhibition of the two transport processes. The quantitative effect of DIDS-binding to the red cell membrane on the bicarbonate-induced Na net flux was examined as shown in Fig. 2. It was found that the inhibition of the Na transport bears the same quantitative relationship to the binding of DIDS molecules to the red cells as has previously been found for anion transport (Lepke et al. 1976, Ship et al. 1977, Funder et al. 1978). Binding of 1.13×10^{6} molecules per cell causes a complete abolition of the bicarbonate stimulated Na net flux.

The anion transport in human red cells is characterized by a pronounced temperature dependence. The apparent Arrhenius activation energy of chloride transport is 20 kcal/mol (=84 kJ/mol) above 15°C (Brahm 1977). The temperature dependence of the bicarbonate-stimulated Na influx was examined in the temperature range between 28 and 38°C. It was found that the overall activation energy of the transport process was 20.8 kcal/mol (=87 kJ/mol) (Table). The apparent activation energy was somewhat lower in the DIDS-treated cells (17.[illegible] kcal/mol) (=7[illegible] kJ/mol). The apparent activation energy of the DIDS-sensitive Na influx, calculated by difference, was 22.1 kcal/mol (=93 kJ/mol). It was not possible to extend the study to lower temperatures due to the pronounced decline of sodium transport with temperature.

The effect of bicarbonate on K, Rb and Cs transport

Passive movements K, Rb and Cs were also studied in the presence of ouabain to eliminate

Table 1 *The effect of bicarbonate on fluxes of Na in normal and DIDS treated human erythrocytes (pH 7.4 38°C ouabain 10 ⁴ M)*

The media contained 150 mM Na as the only cation Chloride was substituted with bicarbonate keeping the sum of anions 150 mM The influx of Na (M_{in} mmol kg cell solids h) without pretreatment of the cells with DIDS was a linear function of the bicarbonate concentration M_{in}=0 1 4×HCO_3+5 7 (correlation coefficient 0 99) In the DIDS treated cells the effect of bicarbonate was abolished and the permeability of the cell membrane to Na was the same as in a 150 mM NaCl medium The results were obtained with erythrocytes from one donor and are the mean values of exp at each experimental condition

mM HCO	0 DIDS M_{in} (mmol kg cell solids h)	M_{out}	M_{net}	+ DIDS M_{in}	M_{out}	M_{net}
0	6.9	4.2	2.7	6.1	3.9	[illegible]
50	10.0	4.0	6.0	5.9	3	2.7
100	18.2	7.9	10.3	5.8	6	3
150	24.8	8.1	16.7	5.8	2.6	3

inhibition of chloride self-exchange and bicarbonate stimulated Na net flux (Fig 1 and 2) were done as described by Funder et al (1978) The same reference gives information about the chemical data of the DIDS preparation DIDS was stored in the dark at −20°C as a dry powder and a fresh solution was prepared for each experiment In a few experiments it was found that potassium influx varied by a factor of two in cells that had been pretreated at a DIDS concentration above 10 DIDS molecules per cell The potassium influxes in DIDS-treated cells were therefore studied after adding DIDS (2×10⁸ molecules per cell) to the suspension 15 min before the experiment was started by the addition of ⁴²K Control experiments showed that anion exchange is inhibited by more than 99% by this procedure

⁸⁶Rb (as RbCl 1 15 Ci/g Rb) ¹³⁷Cs (carrier free as CsCl) ⁴²K (as KCl 1 3 Ci/g K on delivery) from AEK Risø Denmark and ²⁴Na (as NaCl 900 Ci/g Na) were obtained from Radiochemical Centre Amersham England ¹³⁷Cs was dissolved in 1 M HCl which was neutralized with CsOH before use The isotopes were counted by their gamma radiation in a gamma well scintillation counter (Packard Modumatic VI model 5221) 0 1–0 2 μCi/ml cell suspension was used

The flux studies were performed in 150 mM salt solutions of chloride or bicarbonate the cation being either Na K Rb or Cs (Table 4 Figs 1 and) In the experiments with varying bicarbonate concentration (Tables 1 and 3) the media were prepared by mixing the NaCl and $NaHCO_3$-solutions in the proportion giving the anion composition indicated in the Tables pH in these media was kept at 7 4 by adjusting the CO partial pressure to the appropriate value (≤50 mmHg in the 150 mM bicarbonate medium). In the 150 mM K media the concentration of ouabain was increased to 10 M in order to ensure complete inhibition of the active potassium influx

The unidirectional fluxes of sodium (Table 1) were calculated by means of an integrated flux equation based on the linear increase of cellular sodium content during the experiment as described by Funder & Wieth (1967*a*)

Potassium in the cells and the media was determined by flame photometry The net fluxes of potassium could therefore be calculated both from the loss of cell potassium and from the gain of potassium in the media. As the flux values calculated by the two methods were of the same magnitude the figures given in Table 3 are the mean values of the net flux calculated by the two methods The influx of K Rb and Cs (Table 4) was calculated by means of the known extracellular specific activity of the cations and the uptake of the radioactivity over periods during which the radioactivity of the cell increased linearly with time (3 hours)

The mean apparent activation energy E in the temperature interval from 38 to 28°C (Table 2) was calculated from the equation (Exner 1964)

$$E=\frac{RT_1T_2}{T_1-T_2}(\ln J_1-\ln J_2)$$

where T_1 and T_2 are the upper and lower absolute temperature R is the gas constant and J_1 and J_2 are the Na influxes at the two temperatures All fluxes are expressed in units of mmol kg cell solids h The fluxes can easily be related to membrane area because 3 1×10¹⁰ cells the number of normal cells containing one kg cell solid have a membrane area of 4 4×10 cm²

RESULTS

The role of the anion transport system for bicarbonate stimulated Na transport

Unidirectional fluxes of Na were studied in ouabain treated human red cells suspended in electrolyte media with increasing concentrations of bicarbonate The results are shown in Table 1 and they confirm the effect of bicarbonate on Na influx into ouabain treated cells (Funder & Wieth 1967*b*) A gradual increase of bicarbonate concentration

4 4 -diisothiocyanostilbene 2 2 -disulfonic acid (DIDS)

which can be transported by the anion exchange mechanism. This ion pair hypothesis which has recently been reviewed by Lew & Beaugé (1978) received support when Callahan & Goldstein (1972, 1978) found that the HCO_3^- induced Na influx is sensitive to 4-acetamido-4'-isothiocyano-stilbene disulfonic acid (SITS) a potent inhibitor of anion transport (Knauf & Rothstein 1971) Similar observations on the inhibitory effect of anion transport inhibitors on HCO^- stimulated Li fluxes were made by Duhm & Becker (1977) Funder et al (1978) established the quantitative relation between inhibition of anion transport and of the HCO_3^- stimulated Li net flux. The inhibitor used was DIDS which so far is the inhibitor with the highest specificity for the transport system (Ship et al 1977)

The purpose of the present study was to examine whether also the bicarbonate-stimulated sodium permeability can be correlated to the functional capacity of the anion transport system. Moreover it was examined whether bicarbonate affects the permeability to K Rb and Cs which are not assumed to form ion pairs with carbonate

Sodium transport

It seems likely that the increase of Na influx which is seen when chloride in media and cells is substituted with bicarbonate is caused by the transport of $NaCO_3^-$ The effects of DIDS treatment of the cell membrane on Cl^- self-exchange and on N net flux (Fig 1) clearly indicates that the DIDS-sensitive Na flux is linearly correlated to the inhibition of Cl self-exchange A complete (i.e 99%) inhibition of Cl self-exchange is accompanied by total abolition of the bicarbonate-stimulated Na influx Funder et al (1978) demonstrated that inhibition of bicarbonate-stimulated Li net flux is linearly related to DIDS-binding to the erythrocyte membrane Fig. 2 demonstrates that the same relation holds for inhibition of bicarbonate-stimulated Na net flux and it is, therefore likely that both Li and Na can be transported by the anion exchange system in the presence of HCO_3^-

One might anticipate that the transport of $NaCO^-$ will vary with pH because the concentration of ion pairs varies linearly with carbonate concentration However interpretation of the pH dependence of the bicarbonate-mediated Na transport is complicated by the variation of anion transport with pH Thus Cl self-exchange in red blood cells decreases both above and below pH 7.., where it has a well defined maximum at 38°C (Brahm 1977) The HCO_3^- stimulated Na influx has been found to double (from 10 to 22 mmol kg cell solids h) when pH is increased from 7 1 to 7 7 in a 142 mM $NaCO_3^-$ medium (Wieth & Funder 1963) This pH variation causes a fourfold increase of $NaCO^-$ concentration but at the same time anion exchange decreases by 30% between pH 7.2 and 7.8 (Brahm 1977) making a quantitative assessment difficult. It is impossible to examine Na influx at low pH values in the presence of 150 mM HCO_3^- because the pH is 6 94 when the medium is saturated with CO_2 (P_{CO_2}=720 mmHg) It has therefore been very valuable that Becker & Duhm (1978) have reported that a number of oxy anions other than CO appear to form ion pairs by a mechanism similar to that postulated for CO_3^{2-} Because oxalic acid is a relatively strong acid with a pK of 3.8 the concentration of ion pairs does not vary in the interesting pH range between 6 and 7 Becker & Duhm (1978) found a 70% decrease of oxalate-induced Li uptake when pH was lowered from 7 4 to 6 Also in agreement with the studies of anion exchange in the red blood cells they found a slight decrease at pH values from 7 4 to 8 4 although it must be noted that the de

Table 4 *Influx of K Rb and Cs from 150 mM chloride or bicarbonate media into normal and DIDS-treated red cells*

The amounts of DIDS used caused more than 99% inhibition of chloride self-exchange and of bicarbonate-induced N and Li influx. The ouabain concentration was 10^{-4} M in Rb and C media, 10^{-4} M in the K media, pH 7 4 38°C At each experimental condition 6 expts. were performed with red blood cells from 3 donors

150 mM	0 DIDS (mmol kg cell solids h)	+ DIDS
KCl	4.2 (5 0–3.5)	4 1 (4.3–3 7)
$KHCO_3$	4 4 (5 6–3 1)	3.3 (3 6–2 7)
RbCl	4 9 (6 0–4 1)	5.3 (6 3–4 6)
$RbHCO_3$	4.8 (5 4–4.3)	4 9 (6.1–3 7)
CsCl	4.3 (5.3–3 4)	4.5 (6 2 3.4)
$CsHCO_3$	4.3 (3 4–3.9)	4 0 (4.7 [illegible])

Table 2 *Temperature dependence of Na influx into normal and DIDS treated human erythrocytes suspended in 150 mM $NaHCO_3$ containing media (pH 7 4 ouabain 10 $^{-4}$ M)*

The results for Na influx are given as the mean values of 2 expts at each temperature with cells from donors. The apparent activation energy is calculated as stated in the Methods section

Temperature (°C)	Sodium influx (1) Untreated red cells (mmol kg cell solids h)	( ) DIDS-treated red cells	(1–2) DIDS-sensitive sodium influx
38	4 8	5 8	19 0
28	8 1	2.3	5 8
Apparent activation energy (kcal/mol)	20 8	17.2	22 1

active transport of the cations The net loss of K from red blood cells suspended in media containing 150 mM Na and from zero to 150 mM HCO_3 (Cl being the substituting anion) is shown in Table 3 There was no significant effect of increasing the HCO_3 concentration of the medium on the net loss of K At a concentration of zero mM HCO_3 the K net flux was on an average 3 3 mmol kg cell solids h $^{-1}$ to be compared with a net flux of 4 0 in the presence of 150 mM HCO_3 and this net flux was not reduced by DIDS treatment of the cells The study also showed that there was no effect of DIDS on the K loss at lower concentration of HCO_3 evidenced by the fact that the K net fluxes in the presence and absence of DIDS

Table 3 *The lacking of effect of bicarbonate on the net flux of K from normal and DIDS treated erythrocytes incubated in media containing 150 mM Na (pH 7 4 38°C ouabain 10 M)*

Substitution of chloride with bicarbonate or treatment with DIDS did not change the permeability of the cell membrane to K The results were obtained from the experiments reported in Table 1

mM HCO	0 DIDS M_{net} (mmol kg cell solids h)	+ DIDS M_{net}
0	3.3	9
50	3 7	3.8
100	4 6	4 9
150	4 0	4 0
Mean	3 9	3 9
S D	0 65	0.56
n	8	8

at each HCO_3 concentration were almost identical

Passive movements of K Rb and Cs were studied by incubating erythrocytes in 150 mM solutions of the chloride and bicarbonate salts of the three cations Table 4 shows that there was no significant increase of the influx of the cations when 150 mM Cl was substituted with 150 mM HCO_3 It was also clear that DIDS treatment of the cells did not change the magnitude of Rb and Cs influx in Cl and HCO_3 media K influx was unaffected by DIDS treatment in the Cl medium In the HCO medium there was a reduction of the influx from 4 4 to 3 3 mmol kg cell solids $^{-1}$ h after the DIDS treatment of the cells and the K influx in the DIDS-treated cells was also lower in the HCO_3 medium than in the Cl medium

DISCUSSION

Interest in the effect of HCO_3 on Na transport through the erythrocyte membrane was prompted by the clinical observation that patients with metabolic alkalosis characterized by an increased HCO concentration in the plasma accumulate Na in their erythrocytes (Funder & Wieth 1967*a, b*) In vitro studies showed that the Na uptake was caused by an increased passive permeability to Na (Funder & Wieth 1967*b*) and that the active transport of Na was not affected in the presence of HCO (Wieth 1969) Wieth (1970) observed that both Li and Na permeabilities are affected and proposed that the increased permeabilities were due to the formation of the ion pairs LiCO and NaCO

hich can be transported by the anion exchange mechanism. This ion pair hypothesis which has recently been reviewed by Lew & Beaugé (1978) received support when Callahan & Goldstein (1972–1978) found that the HCO_3^- induced N influx is sensitive to 4-acetamido-4'-isothiocyano-stilbene 2,2'-sulfonic acid (SITS), a potent inhibitor of anion transport (Knauf & Rothstein 1971). Similar observations on the inhibitory effect of anion transport inhibitors on HCO_3^- stimulated Li fluxes were made by Duhm & Becker (1977). Funder et al. (1978) established the quantitative relation between inhibition of anion transport and of the HCO^- stimulated Li net flux. The inhibitor used was DIDS which so far is the inhibitor with the highest specificity for the transport system (Ship et al 1977).

The purpose of the present study was to examine whether also the bicarbonate-stimulated sodium permeability can be correlated to the functional capacity of the anion transport system. Moreover it was examined whether bicarbonate affects the permeability to K, Rb and Cs which are not assumed to form ion pairs with carbonate.

Sodium transport

It seems likely that the increase of Na influx which is seen when chloride in media and cells is substituted with bicarbonate is caused by the transport of $NaCO_3^-$. The effects of DIDS treatment of the cell membrane on Cl^- self-exchange and on Na net flux (Fig. 1) clearly indicates that the DIDS-sensitive Na flux is linearly correlated to the inhibition of Cl self-exchange. A complete (i.e. 99%) inhibition of Cl^- self-exchange is accompanied by total abolition of the bicarbonate-stimulated Na influx. Funder et al (1978) demonstrated that inhibition of bicarbonate-stimulated Li net flux is linearly related to DIDS-binding to the erythrocyte membrane. Fig. 2 demonstrates that the same relation holds for inhibition of bicarbonate-stimulated N net flux and it is, therefore, likely that both Li and Na can be transported by the anion exchange system in the presence of HCO_3^-.

One might anticipate that the transport of $NaCO_3^-$ will vary with pH because the concentration of ion pairs varies linearly with carbonate concentration. However, interpretation of the pH dependence of the bicarbonate-mediated Na transport is complicated by the variation of anion transport with pH. Thus Cl self-exchange in red blood

Table 4 *Influx of K, Rb and Cs from 150 mM chloride or bicarbonate media into normal and DIDS-treated red cells*

The amounts of DIDS used caused more than 99% inhibition of chloride self-exchange and of bicarbonate induced Na and Li influx. The ouabain concentration was 10^{-4} M in Rb and Cs media, 10^{-4} M in the K media, pH 7.4, 38°C. At each experimental condition 6 expts. were performed with red blood cells from 3 donors

150 mM	0 DIDS (mmol kg cell solids h)	+ DIDS
KCl	4.2 (5.0–3.5)	4.1 (4.3–3.7)
$KHCO_3$	4.4 (5.6–3.1)	3.3 (3.6– 7)
RbCl	4.9 (6.0–4.1)	5.3 (6.3–4.6)
$RbHCO_3$	4.8 (5.4–4.3)	4.9 (6.1 3.7)
CsCl	4.3 (5.3–3.4)	4.5 (6– 3.4)
$CsHCO_3$	4.3 (5.4–3.9)	4.0 (4.7–.8)

cells decreases both above and below pH 7.2 where it has a well defined maximum at 38°C (Brahm 1977). The HCO^- stimulated Na influx has been found to double (from 10 to 22 mmol kg cell solids h) when pH is increased from 7.1 to 7.7 in a 142 mM $NaCO_3$ medium (Wieth & Funder 1963). This pH variation causes a fourfold increase of $NaCO_3^-$ concentration but at the same time anion exchange decreases by 30% between pH 7.4 and 7.8 (Brahm 1977) making a quantitative assessment difficult. It is impossible to examine Na influx at low pH values in the presence of 150 mM HCO_3^- because the pH is 6.94 when the medium is saturated with CO (P_{CO} =720 mmHg). It has therefore been very valuable that Becker & Duhm (1978) have reported that a number of oxy anions other than CO_3^- appear to form ion pairs by a mechanism similar to that postulated for CO^-. Because oxalic acid is a relatively strong acid with a pK_a of 3.8 the concentration of ion pairs does not vary in the interesting pH range between 6 and 7. Becker & Duhm (1978) found a 70% decrease of oxalate-induced Li uptake when pH was lowered from 7.4 to 6. Also in agreement with the studies of anion exchange in the red blood cells, they found a slight decrease at pH values from 7.4 to 8.4 although it must be noted that the de-

Table 5 *The apparent association constants for the reaction between H^+ Li Na and CO_3^{--} (ionic strength 0 15 M)*

	Association constant M
H +CO ⇌ HCO	6.3×10⁹ (1)
Li +CO ⇌ LiCO	7 4×10¹ (2)
Na +CO ⇌ NaCO	1 8×10¹ (3)

The values are taken from (1) Siggaard-Andersen 1962 (2) Funder et al 1978 (3) Garrels et al 1961 Siggaard-Andersen 196_

crease is considerably less steep than that found for Cl exchange at 38°C by Brahm (1977)

Table 5 shows estimates of the apparent association constants for association between H Li Na and CO_3 The tendency of H to combine with CO_3 is 10^8 times greater than the tendency of Na and Li to form ion pairs with $NaCO_3^-$ However the concentration of Na and Li ion pairs in a 150 mM HCO solution at pH 7 4 will be high enough to explain the HCO_3 induced increase of Na and Li fluxes found even if the permeabilities to the ion pairs are two orders of magnitude smaller than the Cl permeability (Funder et al 1978)

Potassium transport

Garrels Thompson & Siever (1961) and Garrels & Thompson (196_) concluded from electrometric determinations of activity coefficients in carbonate solutions that Na forms ion pairs with CO to a significant degree whereas ion pair formation between K and CO could not be detected Therefore the ion pair hypothesis predicts that the K permeability should not be affected by bicarbonate The results of Table 3 clearly shows that there was no effect of HCO or of DIDS treatment of the cells on the K net flux from red cells into 150 mM Na media The results of Table 4 further demonstrate that the K influx from media containing 150 mM K into red cells was not increased when Cl was replaced with HCO However K influx from the bicarbonate medium decreased slightly but significantly by 1 1 mmol kg cells solids h in DIDS-treated cells It can therefore not be excluded that a small amount of potassium can be transported as carbonate ion pairs However the extra potassium influx is less than 10% of the bicarbonate-stimulated sodium influx shown in Table 1 so the amount transported as ion pairs must be very small In this context it should be noted that Garrels & Thompson (1962) remarks that the electrometric method would not be able to detect minor degrees of ion pair formation because one must accept deviations of up to 10% from the theoretical values of the activity coefficient The present results may suggest that a small fraction of potassium influx is mediated by ion pair formation but the conclusion rests on the assumption that DIDS only affects the anion transport of the red cell membrane Although DIDS has been found to be a highly specific inhibitor of anion transport it can of course not be excluded that the inhibitor may affect other membrane functions

Rubidium and cesium transport

Robinson & Harned (1941) explained ion pair formation between oxyanions and alkali metal cations as a kind of ion solvent interaction where the oxygen atom of the anion replaces an oxygen atom in the hydration shell of the cation (localized hydrolysis) The tendency to ion pair formation decreases with increasing size of the cation predicting that ion pair formation will decrease through the series Li>Na>K>Rb>Cs The hypothesis accordingly predicts that ion pair formation by Rb and Cs is even smaller than with K In agreement with this prediction it was found that neither Rb nor Cs influx was affected by the presence of bicarbonate or by inhibition of the anion transport system with DIDS (Table 4)

Conclusion

The HCO dependent Na transport of the erythrocyte has been shown to be quantitatively related to the functional capacity of the anion transport system suggesting that Na like Li can be transported through the membrane in the form of carbonate ion pairs The mechanism by which red cells incubated in a NaCO medium will take up Na HCO and water is identical to that shown by Funder et al Fig 11 (1978) involving NaCO / HCO exchange through the anion pathway with a subsequent intracellular formation of HCO from CO diffusing into the cells (a mechanism similar to the so-called Jacobs Stewart cycle first proposed by Jacobs & Stewart (194?))

The findings add further support to the concept that the carbonate stimulation of cation fluxes is

specific to Li and Na. Bicarbonate has a negligible effect on potassium influx and no effect was found on the fluxes of Rb and Cs. It seems likely that this specificity can be explained by the theory of localized hydrolysis of Robinson & Harned (1941) because the electric field strength of the alkali metal cations with the larger crystal radii is too small to energize the formation of stable interactions with carbonate ions.

I wish to thank Dr Jens Otto Wieth for many valuable discussions during the performance of this work.

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-e- and postjunctional beta-adrenoceptor mediated fects on transmitter release and effector sponse in the isolated rat portal vein

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Pre and postjunctional control mechanisms of the portal vein of spontaneously hypertensive rats (SHR) were characterized Emphasis was placed on the influence of the presynaptic beta-adrenoceptor mediated mechanism for regulation of neuronal noradrenaline (NA) release (studied as tritium overflow) and its consequences for the contractile response under in vitro conditions. It was found that isoprenaline increased, whereas dl-propranolol decreased the release of neuronal NA during transmural nerve stimulation, while effector responses remained unaltered. d-Propranolol and the beta-1 selective adrenoceptor antagonist, metoprolol, did not affect these two variables. It is concluded that the presynaptic beta-adrenoceptors in the rat portal vein are mainly of the beta-2 type and mediate facilitation of neuronal transmitter release and that concomitant changes of the effector responses of this tissue are below the level of detection under the present experimental conditions.

Key words Autacoid, portal vein, presynaptic beta-adrenoceptor propranolol, metoprolol transmitter release effector response

A variety of autacoids have been found to control the amount of transmitter released per impulse from adrenergic nerves (see Westfall 1977 for review). Noradrenaline appears to modulate its own release via two local feed-back mechanisms mediated via presynaptic adrenoceptors. Thus prejunctional alpha-adrenoceptors mediate a negative feed-back mechanism (Farnebo & Hamberger 1971 Kirpekar & Puig 1971 Langer et al 1971) whereas prejunctional beta-adrenoceptors mediate a positive feed-back control (Langer et al. 1974 Adler-Graschinsky & Langer 1975). The major emphasis in most studies on prejunctional control mechanisms is placed on changes in transmitter release *per se* (see Starke 1977 Langer 1977) and less attention has been paid to the functional consequences for the effector response of altered amounts of noradrenaline (NA) being released per impulse.

In a previous study (Åblad et al 1970 Dahlöf et al 1975) on the vascular resistance of the perfused hind limb of the cat we found that propranolol decreased NA release during sympathetic vasomotor nerve stimulation by up to 30% whereas the vasoconstrictor response was reduced by 15%

The aim of the present study was to characterize adrenergic prejunctional control mechanisms in an isolated vascular preparation the portal vein of spontaneously hypertensive rats (SHR). The main emphasis was given to the influence of the presynaptic beta-mediated mechanism for the regulation of neuronal NA release and its consequences for the contractile response

Two types of experiments were performed First, the effects of dl-propranolol on the contractile responses to transmural nerve stimulation (TNS) at graded frequencies and to exogenous NA were quantitated Second the portal veins were preincubated with ^{3}H 1-NA and the effects of various com-

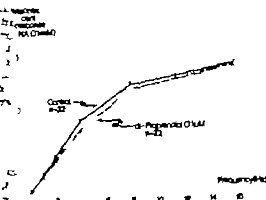


Fig. 1. Excitatory response of rat (SHR) portal vein to transmural nerve stimulation for 1 min at graded impulse rates. Values obtained before and after exposure to *dl*-propranolol (0.1 µM). Responses expressed as per cent of maximal responses to exogenous NA (100 µM). Means ± SE, n = 22.

Statistics

Statistical evaluation of the results was done by Student's t test. Mean values were considered statistically significant when *P*-values were less than 0.05.

RESULTS

Effect of dl-propranolol on the contractile response to TNS at graded frequencies and to exogenous NA

The effector responses to TNS at graded frequencies expressed as a percentage of maximum response to exogenous NA (100 µM) first studied in the control situation and subsequently during exposure to propranolol (0.1 µM) are shown in Fig. 1. The maximum response to TNS before addition of propranolol was obtained at 16 Hz and amounted to 91±1% (n=22) of that produced by exogenous NA (100 µM). In presence of *dl*-propranolol (0.1 µM) there was a tendency to a small shift of the frequency response curve to the right. However, a minimal shift to the right was also noted in the control experiments, where the second frequency response curve was obtained in normal Krebs solution without addition of propranolol. In order to quantitate the effect caused by *dl*-propranolol the changes in response to TNS at each frequency have been expressed as a percentage of the initial response to the same frequency. There was a significant reduction in effector response to TNS only at a frequency of 4 Hz during *dl*-propranolol exposure as compared to control experiments (−12±1.6 and −6±1.9% respectively, $P<0.05$). The maximal mean force response of the portal vein to exogenous NA (13.8±0.5 mN) was not affected by the exposure to *dl*-propranolol (0.1 µM) nor was the maximum peak force value.

In the presence of cocaine, exogenous NA in a concentration of 0.03 µM produced a contractile response which corresponded to about 20% of the maximum response obtained by exogenous NA (100 µM). The vasoconstrictor responses to exogenous NA did not differ in the two experimental groups neither before nor after one group had been treated with *dl*-propranolol (0.5 µM).

Effects on neuronal ^3H-release and contractile response to TNS at 2 Hz

Fig. 2 shows original tracings of contractile force and of integrated force and bars representing ^3H overflow in consecutive 4 min samples from an experiment on isolated superfused SHR portal vein. In the control situation phasic contractions are seen with intervening periods of complete relaxation returning to the baseline of passive force. In response to TNS for 1 min at 4 Hz (S1 = "primer") and at 2 Hz (S2, S3), respectively, the rate and amplitude of the contractions increased to form an incomplete tetanus. The concomitant increase in ^3H-overflow is indicated by the stippled portion of the ^3H-overflow bar. Test substances were introduced at the time indicated, i.e. 16 min prior to the beginning of S3. For details see Methods.

Effects on transmitter release. The average fractional ^3H-release during S2 ranged between $2.7±0.3×10^{-4}$ (n=4) and $4.0±0.01×10^{-4}$ (n=6) in the various series of experiments. In Fig. 3a and b the effects of the tested substances on fractional ^3H-release are expressed in terms of the response to S3 in comparison with that to S2 from individual values obtained in each series. In the control experiments the fractional ^3H-release during S3 compared to that during S2 was reduced by 7±3% (n=9). This change was taken into account when the statistical evaluation of the effects of the tested compounds was performed (see Methods).

Fig. 3 shows the effects of phenoxybenzamine (PBA, 1 µM) and tetrodotoxin (TTX, 0.8 µM). PBA elicited a marked increase of fractional ^3H-release by 473±53% ($P<0.001$). After TTX on the other hand, the ^3H-release response to TNS was practically abolished.

The beta-adrenoceptor active compounds

pounds on transmitter release were studied as fractional ^{3}H-overflow and related to the simultaneously recorded contractile response during TNS

MATERIAL AND METHODS

Spontaneously hypertensive rats (SHR) of the Okamoto strain (Møllegaard Hansens avlslaboratorier A/S Denmark) weighing 250–350 g and of either sex were used Arterial blood pressure (BP) was not measured in the *rats used in this study but SHR of corresponding size* from the same supplier had mean BP of 150–180 mmHg when measured via indwelling catheters in conscious unrestrained animals (Ljung unpublished observations) *The rats were sacrificed by decapitation* The portal vein was dissected free of surrounding tissue and incised to form a longitudinal strip which was tied at both ends with fine silk and mounted under 5 mN passive force in an organ *bath for isometric recording of contractile force by means* of a Grass FT 03 transducer on a Grass polygraph The output signal was electronically integrated over 1 min periods and the integral mean force was recorded on a *separate polygraph channel An induced response* was quantitated as the average mean force over the period of stimulation minus the average mean force developed spontaneously during the preceding 3 min control period *All tissues were allowed to equilibrate in the Krebs solu*tion (see below) for 1 h before the experiment was started

Transmural nerve stimulation was performed by applying square wave pulses of 0 8 ms duration and 200 mA *current in alternate directions* This current was shown to be of supramaximum intensity in separate experiments where the contractile responses to graded currents were studied The pulses were delivered by a constant current *stimulator (Elg to be published) between two electrodes* consisting of rectangular sheets of platinum 7 mm apart placed on either side of the preparation The current density of the field was estimated to be 400 mA/cm^2 Due to the design of the biphasic stimulator electrolysis at the electrodes was minimized The experimental procedure and protocol were as follow

Effects of propranolol on responses to TNS at graded impulse rates and to exogenous NA

Two portal vein preparations were mounted in the same organ bath One min periods of TNS were applied sequentially at 10 min intervals at the frequencies of 1 2 4 8 and 16 Hz respectively After 30 min during which three rinses of the bath were performed the same sequence of stimulations was repeated The effect of *dl*-propranolol 0.1 μM on the frequency response relationship was tested in 22 preparations which were continuously exposed to the antagonist added to the bath solution 20 min prior to the beginning of the second set of TNS applications The neurogenic effector response was expressed as percentage of the maximum response to exogenous NA *100 μM* added to the medium at the end of the experiment

In one set of experiments the effect of *dl* propranolol on responses to exogenous NA was analyzed Cocaine (3 μM) was added to the Krebs *solution during the accom*modation period and maintained throughout the experiments to minimize neuronal uptake Noradrenaline [illegible] μM was applied during six three min long exposure periods at 30 min interval Immediately after the NA exposure *dl propranolol (0 5 μM)* was added to [illegible] of 8 tissues studied and kept in the solution during the remainder of the experiment Each response value was expressed as a percentage of the average obtained for *the initial three NA control responses*

Pharmacological effects on transmitter release and effector response

The portal vein preparation was incubated for 30 min in Krebs solution containing 0 9 μM ^{3}H l NA, then rinsed four times with NA-free Krebs solution and mounted in a 1 ml superfusion bath During the subsequent 1 h wash-out period the preparation was superfused with Krebs solution at a rate of 1.5 ml × min[illegible] Initial stimulation period (S1 = 480 pulses at 4 Hz) [illegible] after the end of the ^{3}H NA incubation was used only to "condition" the tissue and the ^{3}H output and effector responses were disregarded The second and third stimulation period (S2 and S3 = 240 shocks at [illegible] Hz) were applied 24 and 48 min after S1 respectively The activities of the superfusate fractions collected over 4 min periods and of the tissue content after digestion in 1 ml Soluene 100 (Packard Instr) were determined by counting in 10 ml Dimulene (Packard Instr) in a *Packard Tri-Carb* Scintillation Spectrometer The fractional increase in tritium overflow per impulse was calculated as

^{3}H efflux induced by stimulation − spontaneous ^{3}H efflux

Total ^{3}H in tissue at the beginning of stimulation × Number of stimulation impulses

Values for ^{3}H release and effector response during the third stimulation period (S3) were expressed as a percentage of those obtained during the second stimulation period (S2) The average percentage control values obtained during S3 served as a base for the statistical evaluation of drug induced changes of responses to S3 The various substances tested were added to the perfusion medium 16 min before S3 Results are presented as mean ± S E

Solution and drugs

The modified Krebs solution used in all experiments had the following composition in mM NaCl 122 KCl 4[illegible] $CaCl_2$ 2.50 $MgCl_2$ 1 19 $NaHCO_3$ 15 5 KH_2PO_4 1 19 glucose 11.5 and $CaNa_2$ versenate 0 026 It was bubbled with 3% CO_2 in O_2 giving a pH of 7 4 The temperature was kept at 38°C The following drugs were used.

Noradrenaline (l-arterenol bitartrate Sigma Chem Co) l ^{3}H-noradrenaline spec act 5 85 Ci × mmol[illegible] (NEN) *isoprenaline hydrochloride dl-propranolol hy*drochloride and *d* propranolol hydrochloride (ICI) *dl*-metoprolol bitartrate (Hässle) phenoxybenzamine hydrochloride (SK & F) tetrodotoxin (Sigma Chemical Co) Stock solutions in 0 01 M HCl or saline were diluted in Krebs solution immediately prior to administration

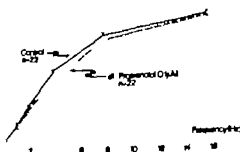


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Pharmacological effects on transmitte release and effector re ponse

The portal vein preparation was incubated for 30 m Krebs solution containing 0 9 µM H l NA It was rinsed four times with NA free Krebs solution mounted in a 1 ml superfusion bath. During the sequent 1 h wash-out period the preparation was fused with Krebs solution at a rate of 1.5 ml × min initial stimulation period (S1 = 480 pulses at 4 Hz) after the end of the H NA incubation was used only condition the tissue and the H output and effector responses were disregarded The second and third stimulation periods (S2 and S3 = 40 shocks at Hz) were applied 4 and 48 min after S1 respectively The activities of the superfusate fractions collected over periods and of the tissue content after digestion Soluene 100 (Packard Instr) were determined by counting in 10 ml Dimilene (Packard Instr) in a Packard Liq Scintillation Spectrometer The fractional increase tritium overflow per impulse was calculated as

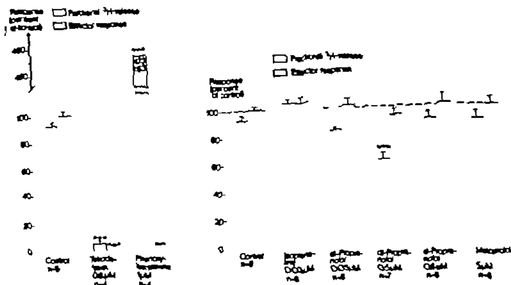
$$\frac{\text{H efflux induced by stimulation} - \text{spontaneous H efflux}}{\text{Total H in tissue at the beginning of stimulation} \times \text{Number of stimulation impulses}}$$

Values for H release and effector response during the third stimulation period (S3) were expressed as a percentage of those obtained during the second stimulation period (S2) The average percentage control values obtained during S3 served as a base for the statistical evaluation of drug induced changes of responses to S3 The various substances tested were added to the perfusion medium 16 min before S3 Results are presented as mean ± S E

Solution and drug

The modified Krebs solution used in all experiments had the following composition in mM NaCl 122 KCl 4 $CaCl_2$ .50 MgCl 1 19 $NaHCO_3$ 15 5 KH_2PO_4 1 19 glucose 11.5 and $CaNa_2$ versenate 0 026 It was bubbled with 3% CO_2 in O_2 giving pH of 7 4 The temperature was kept at 38°C The following drugs were sed

Noradrenaline (l arterenol bitartrate Sigma Chem Co) H-noradrenaline pec act 5.85 Ci × mmol (NEN) isoprenaline hydrochloride *dl* propranolol hydrochloride and *d*-propranolol hydrochloride (ICI) *dl*-metoprolol bitartrate (H ssle) phenoxybenzamine hydrochloride (SK & F) tetrodotoxin (Sigma Chemical Co Stock solutions in 0 01 M HCl or saline were diluted Krebs solution immediately prior to administration



I Effects of tetrodotoxin and phenoxybenzamine on transmitter release (open column) and effector response (stippled column) of the isolated rat (SHR) portal vein during TNS (S3) at 2 Hz. ** indicates $P<0.001$ when values were compared to corresponding values in control tissues during S3

3 b Effects of isoprenaline, dl-propranolol and metoprolol on the transmitter release (open columns) and effector response (stippled columns) of the isolated rat (SHR) portal vein during TNS (S3) at 2 Hz and *** indicate $P<0.05$ and 0.001 respectively when values were compared to corresponding values in control tissue during S3

CUSSION

The responses of the longitudinal smooth muscle of the SHR portal vein to transmural field stimulation at graded impulse rates resembled closely those previously described for portal vein preparations from normotensive rats of the Sprague Dawley strain (Ljung 1970, Johansson et al 1972). Further, since the excitatory responses to transmural field stimulation were abolished by PBA or tetrodotoxin, which demonstrates that responses were selectively elicited via excitation of the intramural adrenergic nerve supply without any direct stimulation of the effector tissue. After PBA weak inhibitory responses to nerve activation at 2 Hz were sometimes noted which might indicate activation of postjunctional beta-adrenoceptors by NA released from the neurons. However neither the effector response to exogenous NA in a low dose (0.03 μM) nor the maximum response to exogenous NA (100 μM) were potentiated by beta-adrenoceptor blockade. This indicates that the alpha-adrenoceptor excitatory neurogenic control of the portal vein of the SHR is hardly influenced by postjunctional beta-mediated effects of NA.

Evoked overflow of tritium after ³H NA preincubation was used to estimate transmitter release. It has previously been shown (Häggendal et al 1970) that in the rat portal vein preparation about 85% of the increase in total tritium overflow induced by TNS at 4 Hz is practically intact ³H NA and that the remainder represents metabolites which in part have been formed after ³H NA release. In the present study there was no evidence for a tetrodotoxin-insensitive release of ³H-NA of neuronal and/or extraneuronal origin as has been found in other tissues (Schrold & Nedergaard 1975). It has not been established to what degree ³H-NA-release reflects the neuronal NA release of the portal vein. However, in experiments in the perfused cat's spleen a proportional release of NA, ³H, H NA and dopamine-beta-hydroxylase occurred over a wide range of transmitter release elicited by low frequency nerve stimulation (Cubeddu et al. 1974). Altogether the discussed observations seem to validate the conclusion that alterations in relative ³H overflow reflect changes in total transmitter release from the adrenergic vasomotor nerves in response to nerve stimulation. This conclusion will be adopted later when the fractional ³H-release in the rat portal vein is discussed.

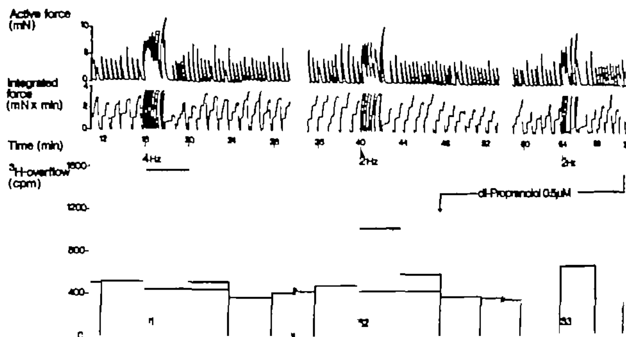


Fig 2 Original tracings of contractile force (upper panel) integrated force (middle panel) and columns (lower pa[illegible] representing ^{3}H-overflow in consecutive 4 min samples from an experiment on isolated superfused rat (SHR) portal [illegible] with *dl* propranolol Open columns represent the spontaneous overflow of radioactivity and the stippled parts indicate increase in overflow above the resting levels produced by TNS The first stimulation period (S1) was used [illegible] to condition the tissue

caused much less dramatic effects on fractional ^{3}H release (Fig 3*b*) Addition of isoprenaline (ISO 0 03 µM) resulted in a small but statistically significant increase of fractional ^{3}H release by 12±3% ($P<0$ 05) Treatment with *dl* propranolol was on the other hand found to decrease the fractional ^{3}H release the reduction being 9±2% ($P<0$ 05) after 0 05 µM and 31±5% ($P<0$ 001) after 0 5 µM *dl* propranolol In contrast to this significant effect of 0.5 µM *dl*propranolol no effect on fractional ^{3}H-release was observed after addition of the same concentration of *d* propranolol Neither was the beta-1 selective antagonist metoprolol (5 µM) found to affect the ^{3}H-release response to TNS

The spontaneous release of tritium from the portal vein preparation was not affected by the different substances tested except for a 3-fold increase produced by NA (100 µM)

Effects on effector response Control TNS during S2 evoked a contractile response corresponding to 27±?% ($n=38$) of the maximal response to exogenous Na (100 µM) the mean values in the different experimental series varying from 26±2% ($n=6$) to 28±2% (n = 8) In the control experiments the effector response during S3 did not differ from that obtained during S2 (Fig 3*a* and *b*)

Fig 3*a* shows that PBA (1 µM) and TTX [illegible] µM) completely abolished the effector response [illegible] TNS A slight inhibitory response to TNS was [illegible] served in 3 of the 6 preparations treated with PBA the reduction of integrated force being less than [illegible]

Fig 3*b* shows how isoprenaline and the [illegible] beta adrenoceptor antagonists influenced the effector response to TNS No significant effect was [illegible] served after isoprenaline (0 03 µM) *dl*-propra[illegible] (0 05 µM) *d* propranolol (0 5 µM) or metoprolo[illegible] µM) After higher concentrations of *dl* propran[illegible] (0 5 µM) however a tendency to a reduction ([illegible] $P=0$ 082) of the effector response to TNS [illegible] obtained

The spontaneous activity of the portal vein [illegible] inhibited by ISO and after PBA treatment by exo[illegible] nous NA (100 µM) These inhibitory respon[illegible] were characterized by an increased rate of sp[illegible] taneous contraction wheras the amplitude of [illegible] contraction was reduced to a relatively grater [illegible] tent The inhibition of the spontaneous acti[illegible] caused by ISO (0 03 µM) led to a reduction [illegible] integrated force by ?0±4% ($P<0$ 01)

Neither ISO nor the beta adrenoceptor block[illegible] in the doses tested affected the maximum respo[illegible] to exogenous NA (100 µM)

— ase in vascular resistance would only corre- 1 to 2% change in smooth muscle cell shorten- according to the law of Poiseuille A change of order would be below the treshold of resolution r most in vitro conditions whether the vaso- tnctor responses are measured isometrically otonically

—tempts to classify the prejunctional beta-ad- receptors according to the beta-1/beta-2 ter ology of Lands et al (1967) have yielded vary- results I the hind limb of the cat the compara- effects of propranolol and the beta-1 selective ker metoprolol indicated that the presynaptic ation of NA release was mainly mediated by 1 receptors (Dahlof et al. 1975) However the lts obtained in studies on isolated blood vessels Stjarne & Brundin (1976) and Westfall et al. 8) indicate that the presynaptic receptors were e beta-2 type This conclusion is supported by present findings with the beta-1 selective an- nist metoprolol This compound did not affect smitter release during TNS when given in a centration producing marked inhibition of the iac chronotropic response to ISO in the isolated atrium (Johansson 1973) We have furthermore xperiments on SHR portal vein with a series eta-1 and beta- selective agonists and antago- obtained results which confirm the existence resynaptic receptors of the beta-2 type (Dahlof l in press) Thus in contrast to the results ob- ed in the anaesthetized cat all discussed in o findings indicate that mainly beta-2 adreno- tors are involved in the mediation of the facilita- of NA release One possible interpretation of data is that the positive control of neuronal release is mediated by presynaptic adreno- tor of both the beta-1 and beta- type with e variations regarding the relative importance ither receptor subtype in different species and ues studied. This type of subreceptor arrange- nt was first suggested by Carlsson et al (1972) characterize postjunctional beta-adrenoceptor diated effects and the hypothesis is supported results from studies of cardiac stimulation rlsson et al 1972) tracheobronchial smooth scle relaxation (Furchgott 1976 Zaagsma & dhof 1976) and adipose tissue lipolysis (Belfrage 7). One factor of interest in this context is the ferential beta-adrenoceptor affinity patterns of agonists NA and A. Since NA has much higher nity to beta-1 than to beta- adrenoceptors and A has relatively more affinity to beta-2 than to beta-1 adrenoceptors, the two catecholamines may elicit a given beta-adrenoceptor mediated effect via different subreceptors (cf Carlsson et al. 1972) This may be of interest when considering phy- siological aspects of hormonal and local neuronal control of presynaptic beta-adrenoceptor mediated feedback mechanism

As regards a hormonal control Stjarne & Brundin (1975) first showed that presynaptic beta-ad- renoceptors could be activated by adrenaline at concentrations similar to those found in plasma in man (cf Vendsalu 1960) In experiments made on the isolated SHR portal vein we found A to be a far more potent agonist for the presynaptic beta-ad- renoceptor mechanism than NA (Dahlof et al 1978 b) This finding supports the suggestion made by Stjarne et al (1975) that beta- adrenoceptors are involved in a physiological control mechanism, through which circulating adrenaline facilitates the release of neuronal NA.

Regarding the local neuronal control of the pre synaptic beta-adrenoceptor mediated mechanism, the NA released may be a significant modulator in certain neuroeffector systems as discussed above However the presynaptic alpha-adrenoceptor mediated feed back mechanism seems to be the major path by which neuronally released NA modulates its own release (Starke 1977) The physiological importance of the local beta-ad- renoceptor mediated positive feed back mechanism remains unclear It has recently been suggested that the adrenergic neuron contains certain amounts of A which is released as a co-transmitter during nerve stimulation to act as agonist to presynaptic beta-adrenoceptors (Rand et al 1978). Further studies are needed to clarify *whether circulating* and locally released A may serve to reinforce the regional effector control of the adrenergic nerve discharge

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The fractional transmitter release per impulse in the SHR portal vein determined in the present experiments at 2 Hz was (3 4±0 3)×10⁻⁴ A similar value was found in portal vein preparations from normotensive rats of the Sprague Dawley strain (Häggendal et al 1970) In a study by Pegram & Ljung (to be published) fractional ³H NA release in portal veins from SHR and from matched normotensive Wistar Kyoto rats was found not to differ The available data thus indicate that fractional ³H release in the SHR portal vein does not differ from that obtained in normotensive rats

PBA treatment caused an almost 5-fold increase of fractional ³H release This effect was apparently due to blockade of the alpha-adrenoceptor mediated negative feedback control of transmitter release However PBA induced inhibition of neuronal and extraneuronal NA uptake mechanisms might be of contributory importance (Starke 1977) The PBA induced increase of transmitter release during TNS at 2 Hz was more pronounced than that obtained in previous experiments where the same experimental conditions but a lower stimulation frequency (1 Hz) was used (Dahlöf et al 1978*a*) This difference in transmitter release might indicate a frequency dependence of the presynaptic alpha adrenoceptor mediated autoinhibition (cf Westfall 1977)

In the present study *dl* propranolol (0.5 μM) was found to reduce the fractional ³H-release during TNS by 31% while ISO caused an increase of the fractional H release by 12% in the SHR portal vein The facilitating effect of ISO is somewhat lower than the increase we obtained by ISO in our previous study where the SHR portal vein was pretreated with PBA desipramine and normetanephrine In those experiments ISO in a comparable dose was found to increase the transmitter release by 25% and was at least as potent as adrenaline (A) (Dahlöf et al 1978*b*) The discrepancy is best explained by the tenet that the increase of transmitter release caused by ISO in the present experiments was counteracted by the presynaptic alpha adrenoceptor mediated autoinhibition *d* Propranolol in contrast to *dl* propranolol in the same concentration did not influence the fractional H release to TNS It has been shown that *d*-propranolol exerts the same membrane stabilizing activity but is about 50 times less active as regards beta adrenoceptor antagonism than *dl* propranolol (Barrett & Nunn 1970) We therefore conclude that the effect of *dl*-propranolol on fractional H-release during TNS is due to inhibition of a presy[naptic] beta adrenoceptor mediated positive fee[dback] mechanism which was first suggested by La[nger et] al (1974) We have recently obtained more [direct] support for this concept in the portal vein pre[para]tion where pre- and postsynaptic alpha-adrenocep[tor] mediated functions as well as neuronal an[d ex]traneuronal NA-uptake mechanism were [blocked] (Dahlöf et al 1978*a*) Under such condition[s ex]ogenous NA was found to increase the frac[tional] ³H release during TNS at 1 Hz and this effect [...] was inhibited by *dl* propranolol which [...] [re]duced the fractional ³H release

In spite of the clear cut reduction of fract[ional] ³H release in the portal vein after *dl*-propra[nolol] very slight reductions of the effector respon[se to] TNS were obtained This apparent discrepancy [be]tween transmitter release and effector resp[onse] may be due to the fact that in the portal vein [...] nerve impulse seems to lead to very high [...] shortlasting local transmitter concentration pe[aks] (Johansson et al 1972) It is an expected con[se]quence of the high local concentrations in tis[sues] with short neurotransmuscular separation like [the] rat portal vein (Booz 1971) that variation of transmitter release per impulse and thus of the [lo]cal transmitter concentration peak even by a fa[ctor] of two would affect the effector response only [to a] minimal extent (Ljung 1976) Furthermore it [was] concluded above that the exitatory neurogenic [con]trol of the portal vein of the SHR is not influen[ced] by postjunctional beta mediated effects Hence [it is] not likely that a possible attenuation of the con[stric]tor response due to reduced transmitter ef[flux] would have been counterbalanced by simultan[eous] antagonism of beta mediated vasodilator respo[nses]

Evidence of a presynaptic beta-adrenocep[tor] mediated positive feedback mechanism operatin[g in] vivo was obtained in a previous study on the [per]fused hind limb of the cat by Dahlöf et al (197[...] [In] that study *dl* propranolol decreased H NA out[put] to sympathetic nerve stimulation by about 30% [...] this effect was associated with a consistent re[duc]tion of the vasoconstrictor response by about [...] These findings could indicate that wider neuro[mus]cular separations prevail in those sections of [the] vasculature which subserve the main resistan[ce to] the blood perfusion and that these sections po[ssess] greater sensitivity to changes in transmitter rel[ease] than the neuromuscular junctions in the SHR p[ortal] vein On the other hand it ma be argued that

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ring behaviour in stochastic nerve membrane models ith different pore densities

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SKAUGEN E. Firing behaviour in stochastic nerve membrane models with different pore densities. Acta Physiol Scand 1980 108, 49–60. Received 3 May 1979 ISSN 000-6772. Institutes of Physiology and Informatics, University of Oslo Norway

A stochastic nerve membrane model ith two-state pore system was investigated by computer simulation in the uniform (space-clamped) case. The model was based upon the Hodgkin–Huxley equations for the giant axon in squid, but where both the maximal membrane conductances and the rate constants were changed systematically. This was done in order to simulate nerve membranes of small axons where both of these parameters are smaller than in squid. It was found that the effects upon the firing behaviour due to a finite number of pores were not greatly affected by changes in these parameters. When the specific injected current was calculated relative to the maximal membrane conductances, the threshold for firing was increased somewhat, and the frequency-current relationship became slightly more linear when the maximal conductances (or pore density) ere decreased, or the rate constants increased. In the discussion it is shown how the results obtained could be applied qualitatively to the firing behaviour of nerve cells, and that firing in small nerve cells should be significantly influenced by the stochastic effects of finite number of pores. Gating currents ere also discussed, and their effects were found to be insignificant in small nerve cells.

former paper the firing behaviour of an isolated patch of nerve membrane (the space-clamped case) has been investigated when the ffects of stochastic fluctuations of the membrane onductances due to a two-state pore system were aken into account (Skaugen & Walløe 1979). The quations used for the model membrane were based pon the equations empirically found for the squid iant axon by Hodgkin & Huxley (the H–H equations) (195). All the membrane parameters including the maximal specific conductances were iven the values used in the H–H equations.

It is only in very small nerve cells, however that we ould expect the effects of these stochastic uctuations to influence the firing behaviour significantly. It is uniformly found that in small nerve bres the density of sodium pores is much smaller han in the squid giant axon (Jack 1975). This probably also gives smaller values of the maximal specific membrane conductances. The effect of this ay be at least partly counterbalanced by smaller ate constants, as will be shown here

In this paper the effects upon the firing behaviour of different values of the maximal specific membrane conductances and the rate constants are investigated. The results are compared to those presented in the former paper and to the original non-stochastic H–H equations

At the end of the paper the possible applications of the results obtained here and in the former paper are discussed. The possible effects of gating currents are also considered

THEORY

The simplest changes of some of the most important constants in the H–H equations at least from a computational point of view are to multiply all the membrane specific conductances (g_{Na}, g_K and g_L) by a factor η and to multiply all rate constants α β by a factor ϕ ($x = h$ m and n) This is done in the "dimensional analysis" developed by Huxley in order to find how the conduction speed depended upon changes in these parameters (Huxley 1959)

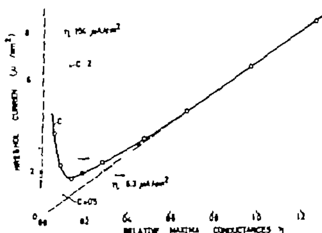


Fig 1 Threshold current as function of the relativ maximal membrane conductances for different values of the membrane capacitance *C* C 0 — C=1 $\mu F/cm^2$ C=0 5 and $\mu F/cm^2$

..er (above threshold) As the stochastic equations ..ere not simulated for η smaller than 0 1 this was ..ot considered important.

The lower broken line in the figure, given by η 6 3 $\mu A/cm^2$ shows the threshold current in ..he case of zero membrane capacitance Inspection ..f Eq (1) shows that, xcept for the injected cur..ent an increase in $\eta = \eta/\phi$ is equivalent to a de..rease in *C* For $\eta = 1$ the threshold current will ..idently approach =6.3 $\mu A/cm^2$ asymptotically ..hen *C* is decreased towards zero It is interesting .. note that for $\eta \geq 0$ 5 the threshold current is close to its asymptotic alue for $C = 0$ For the "normal" case where $\eta = 1$ the membrane capacitance can thus be changed between zero and about twice its actual value of 1 $\mu F/cm^2$ without affecting the threshold current much This insensitivity to the value of *C* has been discussed by Colding-Jorgensen (1976).

Note that the threshold current *i* relative to its asymptotic value always increases as η is decreased, while the absolute alue of reaches a minimum value for $\eta = 0$ 15 It was therefore found convenient to plot the firing frequency as a function

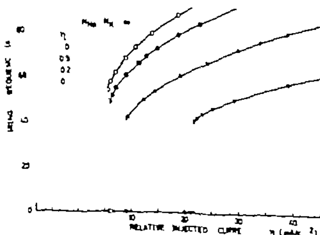


Fig 2 Mean firing frequency as function of relativ injected current $I\eta$ for different values of the relati maximal membrane conductance η when $N_{Na} = N$

He also multiplied the specific membrane capacitance by a factor γ but this parameter will here be assumed constant equal to 1 $\mu F/cm^2$ in all nerve cells considered

The stochastic calculations presented in the preceding paper were based upon the non-stochastic equations (1) and (7) in that paper (these are part of the H–H equations) With the above mentioned scaling these equations become

$$\frac{dE}{d\tau}=-\frac{\eta}{\phi C}(g_{Na}(E-E_{Na})+g_K E+g_L(E-E_L)-I) \qquad (1)$$

$$\frac{dx}{d\tau}=\alpha(1-x)-\beta x$$

where $x=h$ m or n $\tau=\phi t$ and $I=i/\eta$

This set of equations is formally equivalent to Eqs (1) and (7) in the preceding paper where all conductances have been multiplied by the factor $\eta=\eta/\phi$ *since* I *is a free variable and can be chosen at* will In real time t everything will change $1/\phi$ times slower than in these equivalent equations These equations together with the total numbers N_{Na} and N_K of sodium-conducting and potassium-conducting pores completely specify the stochastic equations The numbers N and N_K are obtained from the pore densities and the membrane area of the space-clamped cell under consideration (or part thereof) In order to find the firing behaviour of the space-clamped membrane for all possible combinations of η and ϕ it is thus sufficient to re-calculate the stochastic equations for different values of $\eta=\eta/\phi$ *We may then arbitrarily assume* $\phi=1$ *in* the calculations In all the figures the frequency and current values shown are thus for $\phi=1$ and the value of η shown corresponds to the value of η in the general case where $\phi\neq1$ In order to keep the discussion general we will therefore use η in the text even if we refer to figures where η is used

Since the axon ceases to conduct the action potential when $\eta\leq0.05$ (Huxley 1959 Sabah & Leibovic 1972) the firing behaviour was investigated for $\eta=0.1$ 0.2 and 0.5 The case $\eta=1$ has already been investigated in the preceding paper In this special case where $\eta=\eta/\phi=1$ everything will change $1/\phi$ times slower than shown by the figures in the preceding paper (the frequencies must for instance be multiplied by ϕ) and the scaled injected current I must be multiplied by η in order to obtain the real specific current

But the peak amplitudes and the form of membrane potential changes will not be different

The results obtained in this paper for a certain value of $\eta\neq1$ can also be treated in this way since the model time $\tau=\phi t$ is independent of η The frequencies shown must thus be multiplied by ϕ Instead of the specific injected current i it was found more useful to use the relative specific injected current $I=i/\eta=i\,\phi/\eta$ In the figures the injected current is thus scaled according to the maximal ionic currents in the membrane because these are proportional to η and also scaled according to the rate constants because ϕ is included

In order to compare the results obtained with the non stochastic H–H equations some calculations with these were made in order to find the steady state firing frequency as a function of injected current for different values of η The results are shown in Figs 1 and 2 A method similar to the one described by Hodgkin & Huxley in their paper was used to solve the H–H equations (Hodgkin & Huxley 1952)

The methods used for solving the stochastic equations are described in the former paper (Skaugen & Walloe 1979) The mean firing frequency and an approximate value of the standard error Δf of the mean of this frequency were also calculated as before (Skaugen & Walloe 1979) The value Δf is shown by vertical bars in the figures

RESULTS

Figs 1 and 2 show the results obtained by calculating the response of the original non-stochastic H–H equations to a step injected current for different values of the relative maximal conductances η These equations correspond to the stochastic equations used here but with an infinite number of sodium and potassium pores A step injected current defined by $I=0$ for $t<0$ and $I=I$ for $t\geq0$ was chosen because the frequency-current relationship then includes the lowest frequencies which can be sustained in a steady state firing The threshold current for sustained firing is shown in Fig 1 as a function of η For η smaller than about 0.08 it was difficult to decide whether at a given value of I we had sustained firing or not because the response was a train of action potentials with slowly decreasing amplitudes It was necessary to calculate the response for an excessive long model time in order *to decide whether the amplitudes decreased to zero* (below threshold) or to a constant value larger than

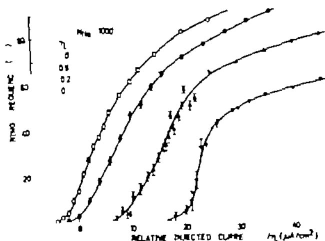


Fig. 5. Mean firing frequency as function of relative injected current I/η for different values of the relative maximal membrane conductance η when $N_{Na} = 1000$ and $N =$

The firing frequency as function of the relative injected current I/η is shown in Fig. 2 for different values of η. As η is decreased, the frequency-current curves are shifted to the right in the figure. This is due to the increase of the relative threshold current. It is noted that the steep (almost vertical) part of the curves at the threshold decreases somewhat in height as η is decreased, but the computations did in all cases indicate an equal abrupt increase of the frequency at the threshold.

Figs. 3 to 6 show the mean firing frequency as function of the relative injected current for different values of η and the pore numbers N_{Na} and N. The curves for $\eta = 1$ are also shown. These were calculated in the former paper (they are there shown in Figs. 4 and 5) and are included here for completeness. As in the former paper the figures show the effects of a finite pore number in one of the two pore systems at a time. In Figs. 3 and 4 the numbers N_{Na} and N of sodium and potassium pores are $N_{Na} = 100$ and N infinite and $N = 100$, N_{Na} infinite, respectively. In both cases the main effect of a reduction of η is to decrease the mean firing frequency f for a given value of I/η. The

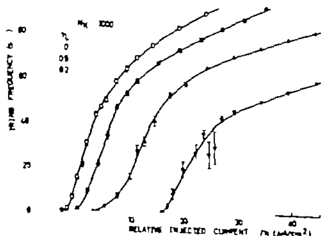


Fig. 6. Mean firing frequency as function of relative injected current I/η for different values of the relative maximal membrane conductance η when N 1000 and $N_{Na} =$

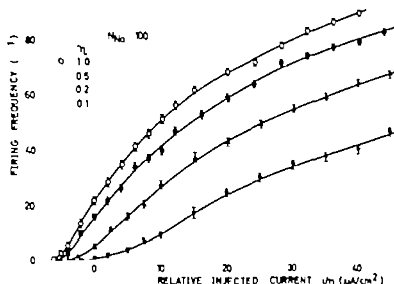


Fig 3 Mean firing frequency a a function of relative injected current i/η for different values of the relati e maximal membrane conductance η when N_{Na}=100 and N =∞

of the relative injected current i/η as this gave a systematic shift to the right of the frequency-current curves when η was decreased If the parameter ϕ is assumed constant this also means that the injected current is shown relative to the maximal ionic currents in the membrane

The upper broken line in Fig 1 given by $i = \eta$ 154 μA/cm² shows the upper limit to the injected current for sustained firing when the membrane capacitance is very small This limit was found analytically by linearisation of the H–H equations (Hodgkin & Huxley 1952) This is valid at this limit because the amplitudes of the action potential then approach zero The triangle bounded by the two broken lines thus shows the combinations of i_T and η where sustained firing is possible when $C=0$ The continuous line shows the region when $C=1$ μF/cm² If C is increased Eq (1) is left unchanged if η and i are increased in proportion This simple scaling makes it possible to find i_T as a function of η for any value of C from the curve for $C=1$ μF/cm² Two examples for $C=0.5$ μF/cm² and for $C=2$ μF/cm² are shown by the dotted curves in Fig 1

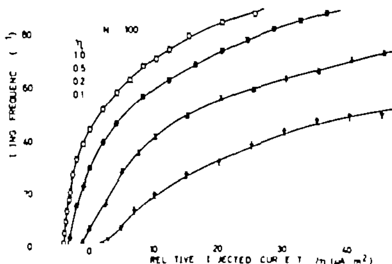
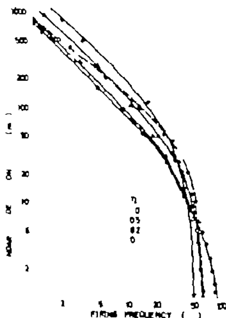


Fig 4 Mean firing frequency as function of relative injected current i/η for different value of the relative maximal membrane conductance η when N =100 and N_{Na}=



8 Standard deviation of interspike intervals as a ...ction of the mean firing frequency for different values ... and for N_{Na}=1 000 (—) and for η=1 and N_{Na}=20 000 ...

...g frequency it was found that a larger value of ...e standard deviation ΔT was connected with a ...reater degree of burst firing either as seen directly ...om inspection of the spike trains, examples of ...hich are given in Fig. or as measured by the ...rst factor *BF* (All these parameters are defined ... the former paper) I do not use the burst factor ... here because ΔT changes in a more systematic ...ty and is easier to interpolate.

The continuous lines in Fig. 8 show the standard ...eviation ΔT as function of the mean firing fre...uency f for different values of η and for N_{Na}= ...000 and N infinite For f smaller than about 40 s ...T increases considerably when η is decreased ...his supports the more qualitative results obtained ...om inspection of the spike trains namely that a ...ecrease in η is followed by an increase in the ...endency to burst firing on the steep lower part of ...e frequency–current relationship For firing fre...uencies f higher than about 40 s the opposite is ...e case But f is now too high to allow burst firing. ... a decrease in ΔT here only means a more regular ...ring Note that this central frequency of f=40 s ... roughly equal to the minimum frequency obtained ...or sustained firing when N_{Na} and N are infinite ...s shown by Fig. 2. This is in accordance with the discussion in the former paper of the mechanisms underlying burst firing.

The broken line in Fig. 8 shows ΔT as a function of f for η=1 N_{Na}=20 000 and N infinite This curve is close to the curve for η=0 1 when N_{Na}= 1 000 and f~20 s which indicates that a reduction of η from 1 to 0 1 gives approximately the same increase in burst firing as an increase of the pore number from 1 000 to 20 000 Corresponding results were obtained for N =1 000 and N_{Na} infinite.

For smaller pore numbers N_{Na}=100 or N =100, there was a small systematic change in ΔT when η was reduced but this change could not be considered significant. There was thus no significant increase in the burst firing when η was decreased at these pore numbers At the same time we note that an increase of the pore number from 100 to 1 000 does not significantly increase the tendency to burst firing when η=1 either This is seen from Figs. 8 and 9 in the former paper

The general conclusion from these results is that assuming ϕ constant, a reduction of the pore densities does not alter the firing behaviour drastically when the pore numbers are kept constant The frequency–current relationship may change somewhat, depending on the number of pores In addition the tendency to burst firing is increased when the pore densities are reduced

Decreasing the pore densities while keeping the pore numbers constant means increasing the area of the nerve membrane which are simulated Another way of looking at these results would be to assume the area constant. A reduction in the pore densities then gives a corresponding decrease in the pore numbers In this case a reduction of the pore densities will not change the tendency to burst firing much according to the discussion above at least not within the range 100 to 10 000 pores If the relative injected current is used, the frequency–current relationship will become less steep and more linear and it will be shifted somewhat towards larger currents This is illustrated in Fig. 9 where the relative pore density η and the sodium pore number N_{Na} are reduced from η=1 0 and N_{Na}=5 000 to η=0.. and N_{Na}=1 000 in the upper part A of the figure and from η=1 0 and N_{Na}= 1 000 to η=0 1 and N_{Na}=100 in the lower part B

DISCUSSION

The effects upon the firing behaviour of a finite number of pores are likely to be greatest in a small

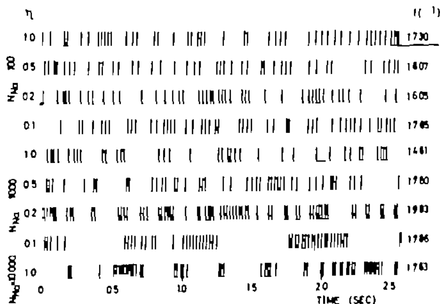


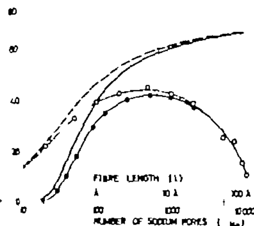
Fig 7 Examples of trains of action potentials obtained for different values of the relative maximal conductances η and the number N_{Na} of Na-pores N =∞

frequency-current relationship becomes less steep (using I/η) and if anything more linear It also takes on a more distinct sigmoid form It should also be noted that while the curves with η =1 are rather different in the two cases shown by Figs 3 and 4 the curves become more similar in shape as η is reduced The standard error of the mean shown by the vertical bars seems to be more or less the same for values of the different parameters

For larger pore numbers the case is somewhat different as shown by Figs 5 and 6 where N_{Na}= 1 000 and N is infinite and N =1 000 and N is infinite respectively The shape of the frequency current relationship seems to be more or less independent of η The steepness of the curves changes very little the only exception is for η =0 1 when N =1 000 and N is infinite but the frequency decreases somewhat when η decreases This latter phenomenom is rather similar to the changes noted in the curves for infinite values of both N_{Na} and N shown in Fig The shift of the curves to the right when η is decreased is also seen but it is somewhat different from that shown in Fig ? For these larger pore numbers (N_{Na}=1 000 and N =1 000) the curves for the same values of η are almost similar for the two pore systems considered sodium in Fig 5 and potassium in Fig 6

For the smallest values of η shown there is also a marked difference in the magnitudes of the standard error of the mean of f as shown by the vertical bars On the steep lower part of the curve they are larger than for larger values of η while for the upper part of the curve they are smaller This is due to a pronounced burst firing on the steep lower part and a quite regular firing on the upper part of the curve This is similar to the firing behavior for even larger pore numbers 5 000–10 000 and above when η =1 as shown in the former paper This is partly illustrated in Fig 7 where typical trains of action potentials obtained in simulation are shown in the case where N is infinite and for different values of N and η The mean firing frequency is in all cases about 17 that is on the steep lower part of the frequency current relationship For N_{Na}=100 there are marked differences to be seen in the spike trains for different values of η But when N_{Na}=1 000 increase in burst firing is seen when η is reduced from 0 2 to 0 1 At the bottom of the figure a spike train for N_{Na}=10 000 and η =1 is shown The tendency to burst firing is also here quite clear perhaps more similar to the case where η =0 1 N_{Na}=1 000 than to the other cases shown

The standard deviation obtained in the simulations can be used to indicate the degree of burst firing We then use the standard deviation ΔT of the interspike intervals which is more precisely defined than the standard deviation of the mean firing frequency shown in the figures For a given mean



g. 10 Mean firing frequency in uniform fibre as function of fibre length in different cases. 100 pores over one space constant. See text for explanation

f the action potential is constant when measured with the scaled time $\tau = \phi t$ which gives $t \sim d^{\cdots} \sim \theta^{\cdots}$ which gives a change in the $t \cdot \theta$ of only 25% over the observed velocity range 6 m/ to 3 0 m/s The product $t \cdot \Theta$ will be closer a constant if ϕ decreases faster than η that is, $= \eta/\phi$ increases when d decreases

The parameters observed, Θ and t are not very sensitive to ϕ and η. *If we for instance assume* $=\eta^{\cdots} \sim d^{\cdots}$ we obtain from Huxley's dimensional analysis $\Theta \sim d^{\cdots}$ and $t \sim \theta^{\cdots}$ against $d^{\cdots}$ nd $\Theta^{\cdots}$ for $\phi = \eta$. At $d = 0.2$ µm this gives $\eta =$ 15 which gives a peak amplitude of 75% of that found at $\eta = 1$. This latter result is in agreement with observations which suggest that the peak amplitude is smaller in small fibres than in large ones.

We see that the scaled H–H equations give results that agree qualitatively with the experiments done on small unmyelinated nerve fibres and that the results obtained here may indicate the effects upon the firing behaviour of small nerve fibres due to a finite number of pores. However it is necessary to establish some connection between the space-clamped, insulated membrane studied here and the physiological operating nerve cell in order to be able to say anything more definite about this. This has been done below. The conclusion is, it is sufficient to find the number of pores over a few space constants.

Let us assume that we are studying the middle part of a very long uniform unmyelinated nerve fibre where the whole fibre is uniformly stimulated by a specific injected current I (The current can be envisaged of as being applied through an axial electrode running along the whole length of the fibre.) In case A a piece with length l of this fibre is insulated at both ends, and the internal resistance of this piece is zero. This is equivalent to the case studied in these papers. The pore numbers are proportional to the length l of the fibre as an example in Fig. 10 one hundred sodium pores are assumed to cover one space constant λ. The mean firing frequency as a function of l will then be as shown by the thin broken line in Fig. 10, which is taken from Fig. in Skaugen & Walløe (1979). The λ does signify the space constant that this fibre model would have had in the presence of a normal nonzero resistance. The specific injected current I is in all cases shown in this figure equal to 5 µA/cm² smaller than the firing threshold in the H–H equations

The thin, continuous line shows case B where a length l of the same nerve fibre is loaded at both ends with the input resistance of the real nerve fibre but where the internal resistance of the piece studied still is assumed zero. The input resistance R_{in} is given by $R_{in} = R\lambda/\pi r^2 = (2\pi r G_m \lambda)^{-}$ where R, G_m, and λ are the internal specific resistance, the membrane specific conductance, the fibre radius, and the space constant $\lambda = \sqrt{r/(2 G R)}$ respectively. This case corresponds approximately to case A, but where the effective injected current is $I_{eff} \simeq I - 2(E_T - E_I)/(A R_{in}) = I - 2\lambda (I - I_T)/l$ where E_T is the threshold potential, E_I is the potential with the injected current $< I$, $A = 2\pi r l$ is the area of the membrane and I and I_T are the specific injected and threshold currents respectively. Figs. and 4 in the former paper were used to find this curve.

The heavy continuous curve shows case C which corresponds to case B but where the internal resistance is taken into account. This is a situation not far from a physiologically operating nerve cell or fibre. This curve cannot be calculated by the methods used here but its form can be indicated qualitatively from a number of its properties.

(*a*) It must approach case B when l is decreased towards zero ($l \ll \lambda$) because the internal resistance then approaches zero

(*b*) For the same length l the mean firing frequency in case C must always be higher than in case B because the internal resistance both makes

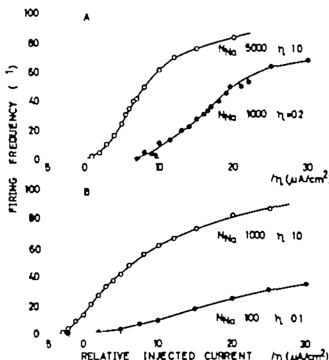


Fig 9 Mean firing frequency as a function of relative injected current I/η for different values of η and N_{Na} Upper part A for $N_{Na}=5\,000\,\eta$ and lower part B for $N_{Na}=1\,000\,\eta$ ○ $\eta=1$ ● $\eta=0._{..}$ (A) and $\eta=0\ 1$ (B)

nerve cell where the total number of pores is much smaller than in a larger nerve cell For the largest nerve cells or fibres such as the squid giant axon we would not expect any effects at all It would thus be of great interest to know if the H–H equations can be used to describe approximately the firing *behaviour in smaller nerve cells because the results* obtained here and in the former paper could then be used at least qualitatively for these nerve membranes also Unfortunately direct measurements of the membrane properties are at present only available for a few large axons but some measurements have been reported mainly of conduction speed Θ but also of rise time for unmyelinated nerve fibres (Jack 1975) It is possible to indicate that these measurements can at least be quantitatively explained by equations of the same form as the H–H equations but with different rate constants and maximal membrane conductances as given by Eq (1)

In order to make discussion simpler we will assume that the pore density ϱ is proportional to η This gives single pore conductances which are independent of the pore densities But this is not necessary in order to use the results presented here because the equations are independent of the actual pore densities or the single pore cond ances In any case it is not likely that single p conductances differ very much between diffe nerve cells as it is believed that the size of pore is an important parameter in its ionic se tivity The observed range of sodium pore dens from 500 pores/μm^2 in the 700 μm thick nerve f in squid to ?.5 pores/μm^2 in the 0 ? μm fibr garfish thus also indicates a value of η 0 0? the latter taking $\eta=1$ in squid (Keynes & R 1974 Colquhoun et al 1972) This small valu η is far below the value $\eta=0\ 05$ where impulse c duction fails if no other parameters are changed

J J B Jack has discussed the probable chan in (specific) membrane parameters in nerve fi of different sizes and he argues that the rate co stants must be smaller in small nerve cells than large ones (Jack 1975) From Eq (1) it is seen t a decrease of η is counterbalanced by a correspon ing decrease of ϕ A small value of ϕ would th make possible a small value of η without suppr impulse propagation A small value of ϕ al accounts for the slow rise of the action potent found in small nerve fibres The relative rate co stant ϕ is probably the only single parameter whi can be changed sufficiently to account for the observations

As a quantitative example we can assume that membrane parameters are power functions of t fibre diameter d This follows the presentation Jack s article from which the data used here a *taken (Jack 1975) Using the observed extremes* fibre diameter d and pore density $\varrho=\varrho_{Na}$ in sq and garfish we find $\eta\sim\varrho\sim_d$?? ($\eta=(d/700\ \mu m)^{?}$ For conduction to take place we must have $\eta/\phi>0\ 05$ at $d=0\ 2\ \mu m$ which gives $\phi<\eta$?? Assuming ϕ to be a certain power of d now dete mines all the non stochastic properties of the fi For instance the conduction speed will be propo tional to $\sqrt{d}$ due to purely geometrical scaling and function of η and ϕ as found in the "dimension analysis" developed by Huxley (1959) This is scaling of the H–H equations similar to that show in Eq (1) but in this case of a propagated act potential In the special case $\phi=\eta$ it gives Θ propo tional to $\sqrt{\phi}$ and then we have $\Theta\sim\sqrt{d}\ \sqrt{\phi}\sim d$ $(d^{??})^{?}=d^{??}$ This agrees reasonably well wi experiments where it is found that $\Theta\sim d^{?}$ to $\Theta\sim d$ in the unmyelinated fibre An approximate i *verse relation between rise time t and Θ has* al been measured In our sc In the tim

guhoun et al 1972) A specific internal resist e $R = 100\ \Omega$–cm and a membrane conductance und the threshold of $G_K = (\rho_{Na}/300$ pores/$\mu m^2)$ mMHO/cm² is assumed. The conductance of n pore is assumed constant and the scaling $\phi = \eta$ sed

or the smallest nerve fibres the stochastic fluc ations of the membrane potential due to a finite mber of pores should significantly influence the ng behaviour The frequency-current relation- p is for instance distinctly more linear for pore nbers up to 20 000 which is much higher than the allest numbers found in this table

Recently a separate gating current has been nd in the nerve membrane in addition to the ic currents (Keynes & Rojas 1974 Armstrong Bezanilla 1974) This current is not explicitly scribed in the H–H equations and its inclusion uld be expected to change the firing behaviour mewhat But since the H–H equations are an mpirical fit to the observed, voltage-clamped be viour the main effects of the gating current must eady be included although not as a separate chanism If the gating current is included as a parate mechanism, the parameters of the original H equations must be changed somewhat in order remove the effects of the gating current from ese In one instance the membrane capacitance C as for instance adjusted down from 1 $\mu F/cm^2$ to 88 $\mu F/cm^2$ (Adrian 1975)

The effects of gating currents connected with the ivation of the sodium conductance can be in uded explicitly in Eq (1) by adding a term C dm/dt where Q is the total charge associated with m-gates per unit area of the membrane If the nductances are multiplied by η and the conduct nce of each pore is assumed constant, the sodium re density and hence Q is also changed by a ctor η At the same time t is replaced by ηt and dm/dt must be replaced by $\eta Q\ dm/dt$ (η is then n outside the parenthesis)

$$\frac{\eta}{\phi}\frac{1}{C}(\ldots(E - E_{Na}) + \phi Q\ dm/dt + g_K E + \ldots (E - E_l)\ldots) \quad (2)$$

This shows that the relative effects of the gating urrent are proportional to ϕ, and thus propor onal to the pore density if the scaling $\phi = \eta$ is used any case this effect will be very slight in small erve cells or nerve fibres It would then perhaps be more correct to use the adjusted value 0.88 $\mu F/cm^2$ for the specific membrane capacitance C in our equations (Adrian 1975) But it would not change the result much as it merely corresponds to a slight ly larger value of η

The latest experiments seem to confirm the existence of a two-state sodium system (Conti et al 1976) But a multistate pore system is still a possi bility especially for the potassium pore system and at any rate in other types of nerve cells. In a three-state pore system, for instance each pore could be shut half open or fully open. This cor responds roughly to the two-state pore system but with twice the number of pores. In general how ever the states in one three-state pore would be coupled and thus it could not be represented by two independent two-state pores In this case separate computations would be needed to find the firing behaviour

The potassium pore system is not so well investi gated experimentally as the sodium pore system, and it may well be that the potassium pores do not operate on an on-off basis, even if it is fairly certain that the sodium pores do. The effects upon firing behaviour due to a finite number of potassium pores will then be less than shown here Hille estimates the potassium pore density to be fifty times larger than the sodium pore density (Hille 1970) and in that case our model predicts a firing behaviour very close to that obtained by assuming an infinite num ber of potassium pores. On the other hand Conti et al. argue that the conductance of a single open potassium pore is three times larger than that of a single open sodium pore (Conti et al. 1975) This gives a potassium pore density ten times smaller than the sodium pore density and the firing be haviour will be dominated by the potassium pore system if its pores operate on an on-off basis

There are several sources of electrical noise in the nerve membrane in addition to the stochastic fluctuations of the membrane conductances con sidered here One example is the thermal noise. These other types of membrane potential fluctua tions will give effects which come in addition to the effects shown here The results presented here thus show lower limits to the expected electrical fluctua tions in more realistic models where all membrane sources of noise have been included According to Lecar and Nossal's discussion however it seems that the potential fluctuations in the node of ranvier are mainly due to sodium conductance fluctuations

Table 1 *Number of sodium pores over two space constants in nerve fibres of different types calc d according to*

$$N_{Na} = \varrho_{Na} \pi d (d/(G_m R_i))^{\frac{1}{2}} = (\varrho_{Na} d^3)^{\frac{1}{2}} \; 6680 \left(\frac{\text{pores}}{\mu\text{m}}\right)^{\frac{1}{2}}$$

Type of fibre	Diameter d (μm)	Sodium pores per μm²	Area over two space constants A (μm²)	Number N_{Na} of sodium pores over two space constants
Squid giant axon [7]	500–900	500	3 4 10⁶–8 1 10⁶	1 7 10⁹–4 1 10⁹
Rabbit vagus nerve [8]	0 3–1 95	?7	1 – 3 510	5 7?0–96 800
Lobster leg nerve [8]	0 2–30 0	16	150–27 500	400–4 4 10⁵
Garfish olfactory nerve [8]	~0 ?	2.5	379	948

the current lost through the load R_1 at both ends smaller and makes the local potential fluctuations in any part of the fibre larger Due to the internal resistance the local fluctuations at any given place in the fibre will be at least slightly different from fluctuations at other places and they will be larger *than the fluctuations of the space mean of the* potential

(*c*) The mean firing frequency f is a monotonically increasing function of l If the nerve fibre of length l is divided in two pieces of lengths l and l_2 each of the pieces will be loaded at both ends with the fibre input resistance R_1 and thus be approximately equal to two separate pieces The combined firing of the two pieces will be higher than the firing in anyone of them but in general f will not be a *linear function of l because the two pieces will* affect each other s fluctuations somewhat When f is increased an increasingly larger part of the time will be spent with one of the pices in a refractory state due to an action potential generated in the other piece When l is increased f will therefore approach a certain limit which in any case cannot be larger than the maximum firing rate of the fibre

Case D is shown qualitatively by the heavy broken curve It corresponds to case A where the fibre is insulated at both ends but with the internal resistance taken into account At any value of l the mean firing frequency f will be larger in case D than in case A because the local potential fluctuations in any part of the fibre will be larger and larger than in case C because there is no current lost through the ends of the fibre As l increases and approaches infinity ($l \gg \lambda$) cases C and D must approach each other and become practically identical because the current lost through the ends in case C will become insignificant compared to the cur through the membrane

In all cases the space constant λ is a very impo tant parameter At $l = \lambda$ the input resistance R equal to the membrane resistance $1/g$ =(G ...) and local fluctuations of the membrane poten *begin to become important* because the intern resistance $R_i l/(\pi r^2)$ then also is equal to $1/g$ Th mean firing frequency f is equal in cases A and (when the fibre length l is in the range l λ (se Fig 10) to a few times λ This relation depe upon the injected current i and the combination pore numbers N_{Na} and N_K used

In a physiologically operating nerve cell t geometry and dimensions around the site for spi generation determines the effects of finite numb of pores upon the firing behaviour *In many ca* this site is part of or connected to the axon r can be represented by a long thin fibre A len of a few space constants of this fibre will acc ing to the discussion above have roughly the sa firing behaviour as the membrane model stud here when the pore numbers are the same We c thus get an idea of the firing behaviour of nerve ce by finding the numbers of pores over a few spa constants in the axon around the site of spike ge ration if known and then u ing the figures p sented here In general both the mean firing f quency f and the specific injected current i s scaled The real values of f and i are found by mu plying the f and i shown in the figures by ϕ a $\eta = \eta/\phi$ respectively

Table 1 shows examples of the total number sodium pores over two space constants for ner fibres of different sizes where the sodium po density has been measured (Keynes & Rojas 19

a Physiol Scand 1980 108 61 71

ie postnatal development of the inferior oblique muscle ' the cat

Fiber sizes and histochemical properties

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Transversal sections of the inferior oblique muscle from kittens of various ages and from adult cats were stained for myofibrillar ATP-ase at Ph 9.4 and 4.35 succinic dehydrogenase (SDH) and fat. The same muscles had previously been submitted to studies of contractional and fatigue properties. With ATPase the fibers could be differentiated into types I, II and IIC. The percentages of each fiber type remained approximately the same from birth onwards. In the inner global layer of the muscle type I fibers showed linear increase in size but types II and II C fibers an accelerated growth after age 70 eeks. In the outer orbital layer here no type I fibers were seen, types II and II C fibers showed the same growth pattern as in the global layer. The fiber content of SHD and fat was low at birth but increased after two week of age. Type I fibers ere poor and type II C fibers rich in SDH and fat. Type II fibers showed varying amounts of these substances. Provided that type I fibers are slow type II fast and type II C intermediate in speed of contraction like in other muscles, the findings on fiber growth and SDH content seem t support the idea that slow fatigue resistant components in eye muscles each maturity earlier than fast components

Key ord Postnatal development, cat extraocular muscle fast and slow fibers histochemistry

has been suggested from physiological data on the natal development f the cat inferior oblique uscle that slowly contracting fibers reach maturity rlier than rapidly contracting fibers (Lennerstrand & Hanson 1978 b). It was thought that histochemical study of developing eye muscles uld further elucidate the time course of the d f entiation of fast and slow fibers. Earlier work on otor unit in hindlimb muscle has shown a close rrelation between the histochemical staining pat ns of the fibers and physiological properties like nt action elocity and resistance to fatigue (Burn m & Kugelberg 1968 Burke et al 1973 Kugelberg 1973). It seems ery likely that this relation holds also for developing hindlimb muscle of kitten (Nystrom 1968 Hammarberg 1975). It has been confirmed in adult rat and cat eye muscles (Hanson & Lennerstrand 1977) but ha not yet been studied in eye muscle of the kitten

In this n estigation the same muscles were examined with histochemical techniques that had been used for the physiological experiment on eye muscle development. Preliminary reports have been published elsewhere (Lennerstrand Hanson & Nichols 1977 Hanson Lennerstrand & Nichol 1977).

METHODS

Histochemical techniques

The physiological experiments on the inferior oblique muscles (IO) of 25 kittens and 3 adult cat were described in previous papers (Lennerstrand & Hanson 1978a b)

(Lecar & Nossal 1971) They did not consider the potassium conductance In the membrane model presented here the potassium conductance fluctuations were also found to be important but this may be at least partly due to the relatively large importance of the potassium current in the squid axon as compared with the myelinated nerve

In addition to the noise due to membrane processes there is also electrical noise in the membrane potential due to synaptic activity Both the quantal release of transmitter substance and the irregular arrival of nervous impulses from other nerve cells or irregular stimulation from sense *receptors will be sources of this "noise This vari*ability in the excitation will in many cases probably be a far more important source of membrane potential fluctuations than the membrane processes themselves especially in large nerve cells but is not considered here The injected current is assumed to be free of any random fluctuations

The space-clamped nerve membrane was chosen as a basis for the model used here in order to simplify the calculations and to be able to compare the results with those of a well-known model But it is of course of more general interest to find how the firing behaviour of a nerve cell in a physiological situation is influenced by a finite number of pores One must then solve the set of equations chosen with both time and space as variables The geometry of the nerve membrane which defines the surface of the cell is then important This has been done for some simple cases and the result will be presented in a later paper

I would like to thank David Attwell and Lars Walloe for stimulating discussions Daniel Kernell and Arild Njå for comments on the manuscript and Torhild Isachsen for drawing the figures Part of the work was done at the University Laboratory of Physiology Oxford It is a pleasure to thank Julian J B Jack and David Whitteridge for their kind hospitality and for providing the computer time needed The work was supported by The Norwegian Research Council for Science and the Humanities

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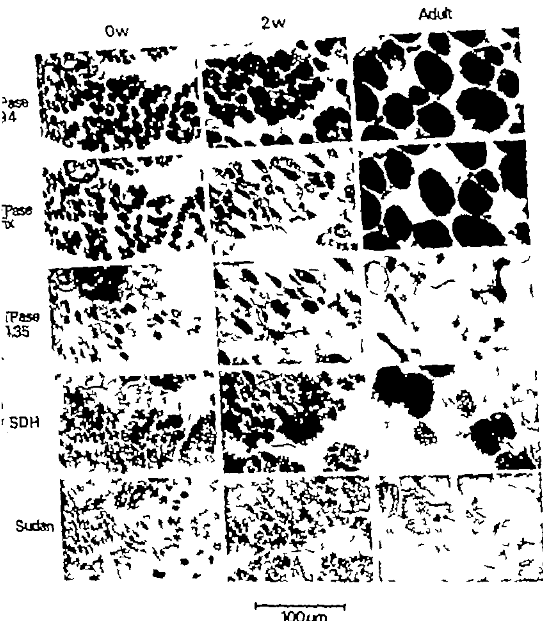
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1. Microphotographs of areas from the global layer in animals of different ages. Consecutive sections stained as ... Note the clear differentiation in fiber types with ATPase stainings already in the newborn kitten. ... SDH and ... the differentiation became clearer ... ith age.

... the adult animal type I fibers were lightly to ... derately stained and orbital type II as well ... s ... bal and orbital typ IIC fibers heavily stained ... lobal type II fibers ... ried from light to dark in ... DH staining intensity. The changes in Sudan stain ... th age and fiber types seemed to parallel those in ... DH activity but with less marked variations.

In a fascicle of the very young muscles stained for ATPase 9.4 the dark fibers were often seen to surround the slightly larger light fibers like the petals of a flower (Fig. 3). Davies (1977) has described a similar arrangement in porcine muscle. It could occasionally be seen also in muscles from older kitten and adult animals but the arrangement be

When tension recordings were completed the muscles were removed and frozen in Freon cooled to −160°C with liquid nitrogen To facilitate handling very small muscles were adhered to a piece of meat prior to freezing. Muscles were stored at −85°C for periods between one week and three months Serial cross-sections (10 μm) cut in a cryostat were taken from the middle third of the muscle close to the nerve entrance

Sections were stained for (1) ATPase after pre-incubation at pH 9 4 (ATPase −9 4) (Padykula & Herman 1955 revised by Brooke & Kaiser 1970) ( ) ATPase after pre-incubation at pH 4.35 (ATPase −4.35) (Brooke & Kaiser 1970) (3) ATPase after prefixation with formaldehyde (ATPase-fix) (Hayashi & Freiman 1966) (4) Succinic dehydrogenase (SDH) (Nachlas et al 1957 Pearse 1960) and (5) Lipids (Sudan Black) (Carleton & Drury 1957)

Measurements performed

The relative area occupied by the global (including when visible the global central) and the orbital layers were estimated (see Alvarado & van Horn 1975) Measurements were made with a planimeter on microphotograph (113×) of the sections stained for ATPase-fix Areas representative of the different layers in each muscle were photographed in all stains Photographs (830×) of samples from the formaldehyde and acid preincubations were printed for determination of stain intensity and fiber size In each sample a random line was drawn across the photograph and 40 consecutive fibers touching this line were graded for stain intensity The two longest fiber diameters at right angles were used for area calculations Cells having one diameter two or more times the other were rejected as not being in cross-section The largest fibers had a greater chance of hitting the line The probability of a fiber touching the line was considered roughly proportional to the sum of the two measured diameters of the fibers. The observed percentages and areas were corrected accordingly The cross-section area of each fiber was calculated assuming it to be ellipsoidal Usually the IO muscles from both eyes were examined Differences between the eyes were assumed to be random

For ATPase-fix and ATPase-4 35 sections average values were calculated in each age group for (1) percentage of fibers which stained darkly and lightly and ( ) average cell area of each of the fiber types Analysis of variance (one way) was used for determining differences between age groups When necessary for a more detailed comparison between age groups confidence intervals were calculated according to the method of Schaffé (1961) In order to determine histochemical profiles of the fibers comparisons of the same 15 to 20 fibers in all stains were made by direct observation through the microscope

RESULTS

Layering of the muscles

In the adult animal the IO muscle is divided into two main parts the *global* layer with large fibers located on the inner global side of the muscle and the *orbital layer* with small fibers located on the outer orbital side (Mayr 1971 Alvarado & van Horn 1975 Hanson & Lennerstrand 1977) [illegible] layers were scarcely discernible at birth and [illegible] not until 10 weeks of age that the layering [illegible] distinct as in adult muscles

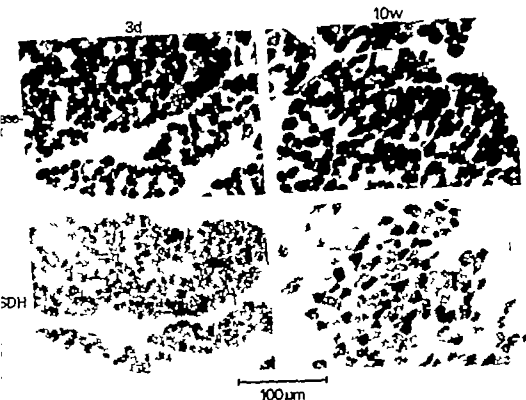
In animals 2 weeks of age and older some bundles seemed to belong to a third global [illegible] layer between the two main layers The fibers [illegible] these bundles were most similar to those of [illegible] global layer although they were slightly smaller [illegible] often arranged more compactly in the [illegible] They have been included in the global layer [illegible] calculating the cross section areas of the different muscle parts

The estimated area of the orbital layer [illegible] from about 20 % of the entire transectional [illegible] the muscles in the young animals to about [illegible] the total muscle area in adult animals As [illegible] previously the layering was not very distinct [illegible] early age and therefore values obtained in [illegible] animals were less exact than those from older [illegible]

General histochemical features and fibers [illegible]

At all ages a high proportion of the fibers [illegible] marked activity of ATPase after incubation at [illegible] 9 4 (with or without pre fixation with formaldehyde) Most of the fibers that were dark at pH [illegible] were left unstained after treatment at pH 4 [illegible] which indicates that they were of type II (Brooke & Kaiser 1970) Such fibers were seen in both [illegible] global and the orbital layers (Figs 1 and [illegible]) [illegible] fibers pale in ATPase 9 4 reversed their color [illegible] ATPase-4 35 and became rather dark This [illegible] cal of type I fibers according to Brooke & Kaiser (1970) These fibers existed only in the global [illegible] (Fig 1) In addition to these two fiber types [illegible] fibers were seen About 1/5 of the fibers in [illegible] orbital layer and 1/10 of the fibers in the global [illegible] stained darkly for ATPase both at pH 9 4 and [illegible] They must be considered type IIC fibers (Brooke & Kaiser 1970)

There was much less differentiation between fibers in SDH activity at birth than in ATPase [illegible] I fibers were pale in SDH with a few large granules with intense color Type II fibers were [illegible] Further differentiation especially in type II [illegible] SDH activity began at about 4 weeks The [illegible] SDH activity of the muscle seemed to increase between [illegible] and 6 weeks of age but the characteristic differences in SDH activity between types I and [illegible] fibers remained the same throughout development



Sections from the global layer stained for ATPase-fix kittens aged 3 days and 10 eeks respectively. The 'flower arrangement of the fibers in ATPase-fix ith dark fibers surrounding the fibers is most marked in the est kitten, here the light fibers are the largest.

types I II and IIC fibers of the global layer types II and IIC fibers of the orbital layer n SDH activity was included in the differentia- the type II global fibers could be subdivided fibers with high and low activ ty. For the other types SDH activity was the same within each p. The significance of this histochemical fiber rentation for the ultrastructural separation of r types in adult cat ey muscle (Alvarado & an n 1975) will be d scussed later. The relative portions of each fiber type determined in con- utiv sections from the same muscles are given Table 1

obal la er fib t pe   nd ue hanges

elat e proportions of fibers with dark and w color   ATPase-4.35 emained fairly constant ing postnatal development (inset of Figs 4 and This was confirmed with an analysis of ariance siz of the type II fibers (dark in ATPase-f x d light   ATPase-4.35) increased stead ly up to 20 weeks of age, followed by an accelerated in- crease between age 20 weeks and the adult stage. Type I fibers which were light n ATPase-fix and dark in ATPase-4.35 increased linearly through the whole age span. Type IIC fibers, dark in both ATPase-fix and -4.35 were not identified and measured separately since it w s possible to trace fibers i both stains only in a few muscles of each age group. The general impression is that IIC fibers were smaller than types I and II fibers and similar in growth pattern to the type IIC fibers in the orbital layer. The proportion of the global type IIC fiber remained the same throughout development.

In the adult cat the type II fiber mean area w significantly larger than type I fiber area ($P<0.05$). At ages below 20 weeks there was no significant difference between the groups.

Orbital lay er fiber types   nd   e changes

All fibers of the orbital layer tained darkly in ATPase-f x in muscles of all ages (Fig. 6 inset)

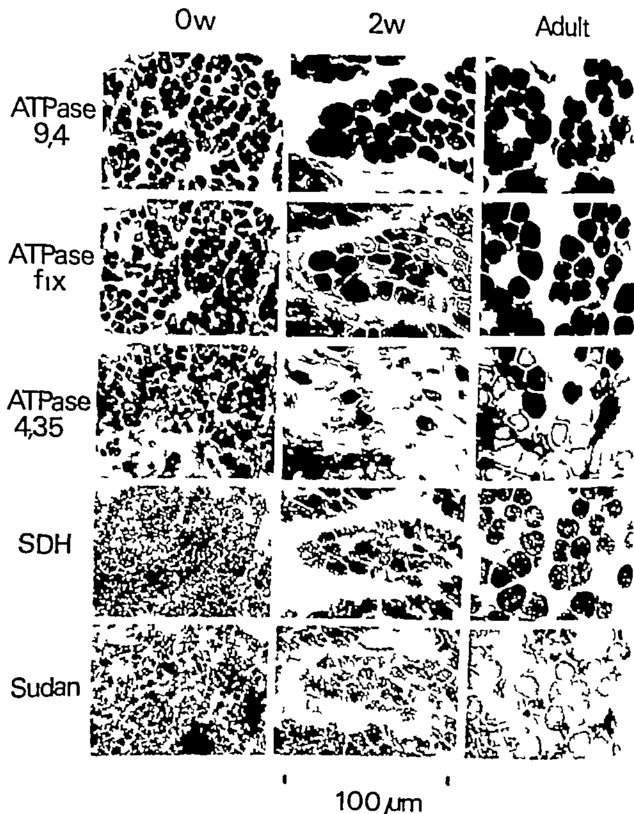
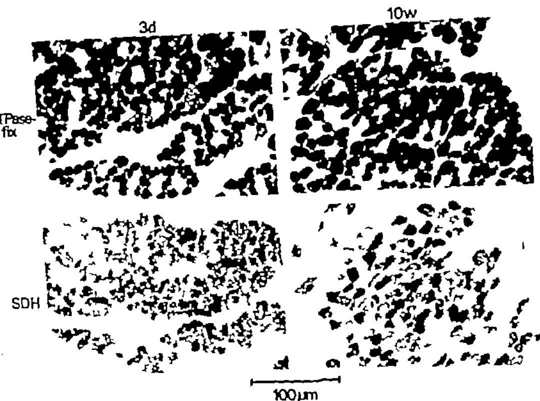


Fig 2 Microphotographs of consecutive sections from orbital reas of the same muscle as n Fig 1 All cells are dark ATPase 9 4 ATPase-fix and SDH In ATPase-4 35 type II and IIC fibers n be d fferentiated already t birth

came less clear with increasing age as the darker fibers grew larger (Fig 3)

In the adult cat it was possible to di tinguish five eye muscle fiber types in the ATPase stainings the distribution of the fibers in the tw layers of th muscle wa also taken into account These type



3 Sections from the global layer stained for ATPase-fi kittens aged 3 days and 10 weeks respectively The 'flower tal arrangement of the fibers in ATPase-fix with dark fibers surrounding the fibers, is most marked in the ungest kitten where the light fibers are the largest

ere types I II and IIC fibers of the global layer nd types II and IIC fibers of the orbital layer When SDH activity was included in the differentia on the type II global fibers could be subdivided nt fibers with high and low activity For the other ber types SDH activity was the same within each roup The significance of this histochemical fiber fferentiation for the ultrastructural separation of ber types in adult cat ye muscle (Alvarado & van orn 1975) ill be discu sed later The relativ oportions of each fiber type determined n con cutive sections from the same muscles are given Table 1

lobal la fiber t pes and si hanges

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Orbital la er fiber types and ize chang

All fibers of the orbital layer tained darkly in ATPase-f in muscles of all age (Fig. 6 inset)

Table 1 *Suggested correlation between ultrastructural and histochemical types of muscle fibers in cat extraocular muscles*

Electron microscopical data from Alvarado & Horn (1975) and Alvarado et al (1978) and histochemical data from present study

EM type	1	2	3	4	5
Layer	Global	Global	Global + orbital	Global	Global + Orbital
Mean diam (μm)	30	22	12	23	1
Membranous SR + TT	Much	Much	Moderate	Poor	Poor
Mitochondria	Few/small	Moderate	Many/large	Few	Many
Proposed innervation	Single	Single	Single	Multiple	Multiple
% of global fibers	38	20	26	15	
% of corbital fibers	–	–	60–70		30–40
Histochem type	Global II	Global II	Global + orbital II	Global I	Global + orbital IIC
ATPase-9 4	++	++	++	0	++
ATPase-fix	++	+ ++	+ ++	0	++
ATPase-4 35	0	0	0 +	++	++
SDH	0	+	++	0 +	++
% of global fibers	36	4	17	14	9
% of orbital fibers	–	–	54		46

Alvarado et al (1979) report occurrence of type 4 fibers in orbital layer and types 5 fibers in global layer but not relative proportions of each type

About 80% of the fibers reversed their staining properties and became pale in ATPase-4 35 being ordinary type II fibers The rest of the fibers were dark also in ATPase-4 35 i e they were type IIC

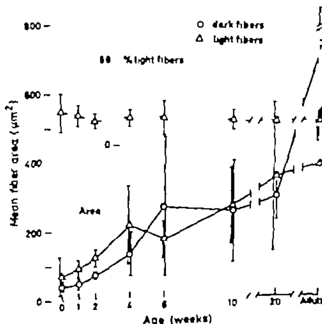


Fig 4 Diagram showing the increase in mean area of global fibers staining darkly (type II and IIC) and lightly (type I) for ATPase-fix The inset show the proportion of light (type I) fibers at different ages The bars indicate standard deviation in this and the following diagram

fibers The proportions types II and IIC fibers did not change significantly with age (Fig 7 inset) The growth patterns were similar in both types of fibers and there were no size differences between types at any age They changed size postnatally in a manner similar to global type II fibers exhibiting the most marked size increase between 20 weeks and adult age after a linear increase up to 20 weeks

DISCUSSION

Differentiation with age in fiber enzyme activity

This study has shown that cat eye muscle can be differentiated into separate fiber types already at birth The relative proportions of the various types did not seem to change with age although the growth patterns were different

In the newborn kitten the fiber differences in staining properties with acid-stable and alkali stable ATPase were as large as in the adult animal Similar differences in the activity of alkali-stable ATPase activity have previously been demonstrated in hindlimb muscle of new born kitten (Karpati & Engel 1967 Nyström 1968) but not in rat and mouse muscles (Dubowitz 1963 1965 Karpati & Engel 1967) Less distinct differences in alkali stable

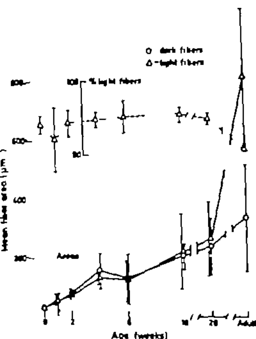


Fig. 5 Graphs to show increase of mean fiber area with age in the *global* layer stained for ATPase-4.35. The dark fibers are type I and IIC, the light fibers type II. The inset shows the percentage of light fibers at different ages

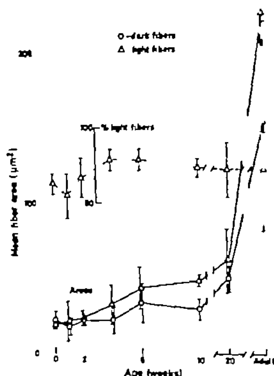


Fig. 7 Changes with age in *orbital* fibers stained for ATPase-4.35. Dark fibers (type IIC) and light fibers (type II) show the same growth patterns. In the inset the percentage of light fibers has been plotted

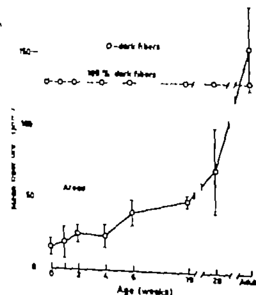


Fig. 6 Graphical presentation of fiber growth in the orbital layer. All fibers stained darkly for ATPase-fix (type II and IIC fibers), as shown by the inset

ATPase activity have been seen in new-born rabbits and hamsters (Dubowitz 1963, 1965). The length of the gestational period has been considered to be an important factor for the muscle fiber differentiation at birth (Dubowitz 1965). However, Ashmore & Doerr (1971) working with chicken demonstrated that the differentiation at hatching varied between muscles and was higher in leg muscles than in the pectoralis muscle.

Acid-stable ATPase activity of developing muscle has not been studied in leg or eye muscles. The present investigation showed that the differentiation was as high in acid-stable as in alkali-stable ATPase activity, which indicates that the development of these enzymes are governed by similar factors. We have also found differences between eye muscle fibers of the newborn kitten in SHD stains, although they were much smaller than in ATPase stains. There was a general increase in SDH-activity after two weeks of age and a further differentiation between fibers in SDH staining

Tabl. 1 *Suggested correlation between ultrastructural and histochemical types of muscle fibers in cat extraocular muscles*

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Histochem type	Global II	Global II	Global + orbital II	Global I	Global + orbital IIC
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ATPase-fix	++	+ ++	+ ++	0	++
ATPase-4 35	0	0	0 +	++	++
SDH	0	+	++	0 +	++
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About 80% of the fibers reversed their staining properties and became pale in ATPase-4 35 being ordinary type II fibers The rest of the fibers were dark also in ATPase-4 35 i e they were type IIC

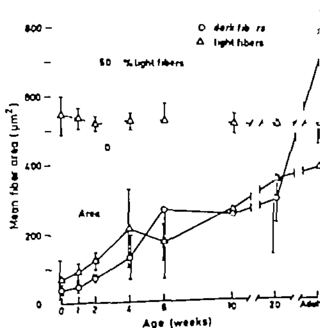


Fig 4 Diagram showing the increase in mean area of *global* fibers staining darkly (type II and IIC) and lightly (type I) for ATPase-fix The inset shows the proportion of *light* (type I) fibers at different ages The bars indicate standard deviation in this and the following diagram

fibers The proportions types II and IIC fibers did not change signficantly with age (Fig 7 inset). The growth patterns were similar in both types of fibers and there were no size differences between types at any age They changed size postnatally in a manner similar to global type II fibers exhibiting the most marked size increase between 20 weeks and adult age after a linear increase up to 20 weeks

DISCUSSION

Differentiation with age in fiber enzyme activity

This study has shown that cat eye muscle can be differentiated into separate fiber types already at birth The relative proportions of the various types did not seem to change with age although the growth patterns were different

In the newborn kitten the fiber differences in staining properties with acid-stable and alkali-stable ATPase were as large as in the adult animal Such differences in the activity of alkali-stable ATPase activity have previously been demonstrated in hindlimb muscle of new born kitten (Karpati & Engel 1967 Nystrom 1968) but not in rat and mouse muscles (Dubowitz 1963 1965 Karpati & Engel 1967) Less distinct differences in alkali-stab

ded that this staining pattern indicated multiple ervation i chicken anterior latissimus dorsi ers with these histochemical properties have o been found in young rat skeletal muscle where y are thought to represent a transition form be een types I and II fibers (Kugelberg 1973). In eye uscle this pattern was certainly not restricted to mature muscle fibers as suggested for skeletal uscle by Guth & Samaha (1972).

Multiply innervated fibers have been shown to e long lasting contractions following applications succinylcholine (Sch) (Bach-y-Rita 1971). How er the amount and also the total transverse sec on of type I and type IIC fibers was too small at all es to account for the whole contraction after h-injection, which usually reached 40-50% of the aximal tetanic tension (Lennerstrand & Hanson 978). Thus either some of the type II fibers are lso multiply innervated or some singly innervated fibers reacted with sustained contraction on appli- tion of Sch.

rrelation between histochemistry and ectron microscopy

lectron microscopy has provided information on he correlation of eye muscle fiber structure and unction (Lennerstrand & Nichols 1977, Bach-y Rita et al. 1977) as well as the relationship of fiber tructure to innervation (Alvarado et al. 1979). His- ochemical studies however take less time and ovide additional independent data on enzymes for iscussion of fiber structure and function. His- ochemistry could be especially useful for survey f fiber types throughout a single muscle. We have ompared the cell types distinguished by his ochemistry with those characterized by electron icroscopy (Alvarado & van Horn 1975). The mul ply innervated fiber types 4 and 5 have already een discussed. They are suggested to be equiva- nt to types I and IIC fibers, respectively. Type I ibers are found in the global layer and type IIC iber in both layers.

Singly innervated fibers of the electronmicro- copical type 3 are small fibers found predominant y in the orbital layer. They are presumably equiva- ent to the orbital type II fibers in histochemistry ype 3 fibers are rich in mitochondria (Alvarado & an Horn 1975) as are the type II fibers, staining arkly for the mitochondria-bound enzyme SDH.

Singly innervated types 1 and 2 fibers are found in he global layer together with type 3, 4 and 5 fibers (Alvarado & van Horn 1975). Both type 1 and 2 are large fibers, type 1 being the largest but containing less mitochondria than type 2. Thus type 1 fibers would seem to correspond best to the histochemical type II global fiber with very little SDH. The type fiber would probably be the histochemical type II global fiber with higher SDH activity.

The information given on the correlation between electron microscopy and histochemistry of the dif ferent eye muscle types has been summarized in Table 1. Classification according to this scheme was difficult in younger cats because of less distinct layering and more random distribution of cell types, as well as smaller size differences between fibers. There were also cells in these preparations which could not be classified. It should be noted that young eye muscles have not yet been examined with the electron microscope.

Functional implications

Demands from the visual and oculomotor systems presumably have a strong influence on the de velopment of cat eye muscles. During the sensitive period of visual development, lasting in the cat from three to about ten weeks of age (Hubel & Wiesel 1970, Blakemore & van Sluyters 1974), motor ac tivity for fixation and binocular fusion would seem of particular importance. These motor functions are probably best subserved by fatigue resistant fibers, for example of type I and IIC. When predatory skills and other complex motor patterns are exer cised at a later age, increased demands for rapid refixations and saccadic eye movements can be ex pected. These might predominantly involve type II fiber activity. There would thus seem to exist func tional justifications for developing slow fiber com ponents earlier than the fast fibers, as suggested from the physiological and histochemical results.

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properties took place. This agrees with previous findings in other kitten muscles (Nyström 1968, Schwieler 1968, Hammarberg 1974).

Correlation between physiology and histochemistry

At all ages the eye muscles contracted much faster than other muscles (Lennerstrand & Hanson 1978a). This is consistent with the comparatively large amount of fast contracting type II fibers present in eye muscles already at birth. Type I fibers would constitute a slow contracting group of eye muscle fibers. The orbital type IIC fibers that stained equally dark for ATPase at pH 9.4 and 4.35 probably contract with intermediate velocity (cf. Kugelberg 1976). These findings are in agreement with the physiological differentiation of eye motor units by Lennerstrand (1974).

Experiments on twitch and tetanic tension development and on the effects of repetitive stimulation were performed on the same muscles that were examined histochemically (Lennerstrand & Hanson 1978a, b). Physiological results suggested that the slow fibers attained their adult properties already at age 6–10 weeks, while the fast fibers seem to reach their mature stage much later. Histological correlates to these differences in functional development might be found in the growth pattern of the 3 fiber types. *Until the age of 10–20 weeks, fibers of all* types were of almost equal size, but after this period growth was much faster in type II and IIC fibers than in type I fibers. A similar relationship has also been demonstrated in cat hindlimb muscles, where the muscle fiber area of the fast gastrocnemius muscle increased considerably more than the fiber area of the slow soleus muscle during the last part of the postnatal development (Eldred & Maier 1974). Since force of fiber contraction is directly related to fiber area, the large late increase in type II fiber size would explain the steady increase in speed of contraction seen in eye muscles up to adult age. The much smaller increase in size of the slow and presumably fatigue resistant type I fibers would be consistent with the finding that fatigue properties changes very little after 10 weeks of age.

The separate time courses for differentiation of ATPase and SDH activities of the muscle fibers indicate that the development of these enzymes are regulated separately. The ATPase is probably governed by neuronal factors other than impulse activity in the motor nerve, while the SDH level would seem adjusted to the amount of work demand[ed of] the muscle and regulated by the flow of impulse[s in] the motoneurons (see for instance Riley & [...] 1973). The opening of the eyelids at 2 weeks of [age] and the development of visual functions at 3[...] weeks would *impose great demands for sustai[ned]* activity in some (not necessarily all) of the [eye] muscle fibers. A concomitant increase of the c[on]traction velocity (Lennerstrand & Hanson 19[78]) probably also augmented the requirements [for] oxidative capacity in some of the eye muscle fi[bers] (cf. Kugelberg 1973). The times at which th[e] claims for fatigue resistance occurred corre[late] well with the observed increase in SDH activ[ity] from two weeks of age and onwards.

Histochemical properties of multiply innervated fibers

Cat eye muscles contain fibers with multiple inn[er]vation, in addition to the singly innervated fib[ers] commonly seen in skeletal muscle (Hess & P[...] 1963, Lennerstrand 1974, Alvarado & van H[orn] 1975, Alvarado et al. 1979). The multiply innerva[ted] fibers of eye muscles are known to be of two ty[pes]. One of them is the fiber in the global layer w[ith] dense multiple innervation, resembling multiply [in]nervated slow fibers in amphibian muscle. Th[ey] were called type 4 fibers in the classification w[ith] electronmicroscopy *by Alvarado & van H[orn]* (1975). It is suggested that the type 4 fibers cor[re]sponded to the histochemical type I fibers found [in] the global layer in this study. Type I fibers contr[act] slowly and are pale in SDH, which i[s] characteristic of amphibian slow, multiply inn[er]vated fibers (Lannergren & Smith 1966, Smith & Lännergren 1968, Smith & Ovalle 1973).

The second type of multiple innervation is repr[e]sented by the orbital fiber of type 5 in the classifica[]tion of Alvarado & van Horn (1975). The end pla[tes] are more widely distributed than on type 4 fibe[rs]. Similar fibers have been found in avian muscles[. It] has been speculated that the fibers with both [al]kali stable and acid stable ATPase, type IIC fibe[rs,] are multiply innervated fibers (Yellin 1969, Ham[marberg] *& Lennerstrand 1977*). The deci[s]ion that the o[rbital fibers] that stained positively in both acid and alkal[ine] pre-incubated ATPase and strongly in SDH [are] equivalent to the type 5 fibers in the classificatio[n of] Alvarado & van Horn is further supported by fi[nd]ings of Asmussen, Kiessling & Wohlrab (19[..,] 1971) and Ashmore & Doerr (1976), who have c[...]

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ɔle of heating in non invasive blood pressure easurements in rats

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BORG E. & VIBERG A Role of heating in non-invasive blood pressure measurements in rats. Acta Physiol Scand 1980, 108:73–75 Received 9 May 1979 ISSN 0001-6772 Department of Physiology II Karolinska Institutet, Stockholm Sweden.

The "tail-cuff" technique for indirect blood pressure measurements was standardized with respect to heating, in experiments on 47 adult Sprague-Dawley rats. The direct, intra-arterial and the indirect blood pressures were simultaneously obtained Pulse volume was recorded from the tail by non-invasive technique, and was controlled by gradual application of heat It was found that pulse volume and body temperature were poorly related A stable relation existed, however between the error in the indirect blood pressure recordings and the pulse volume The error was minimized, provided that the heating was adjusted to induce pulse volume of at least 25% of the maximum obtainable value It was suggested that the validity of the indirect blood pressure recording could be improved, if heat application was regulated on the basis of observations of pulse volume in the tail instead of ambient temperature or body temperature Errors due to e cessive vasoconstriction or discomfort due to overheating could thereby be minimized

Key words Blood pressure, rats, temperature regulation tail

on-invasive measurements of blood pressure in at are commonly made by utilization of the usual tail In principle the technique is equivalent to the one used in humans. The bloodflow is cluded by compression of the tail and the systolic pressure is defined as the level at which ood-flow is just detectable peripheral to the cluding cuff upon elieving the cuff pressure The pacity for detecting a minimal flow is therefore itical to the precision of this technique

One major technical d fficulty and an important urce of measurement error and of data misinterpretation is associated with the recording of ood-flow in the tail. At room temperature the tail teries are constricted the flow is very low and e recording usually has a poor signal-to-noise tio Under such conditions flow will not be detected until the cuff pressure is significantly below e true ystolic pressure In addition, a pressure op will occur in the tail vessel from its origin at e aortic bifurcation to the point of measurement 2 4 cm to the periphery In order to circumvent ese difficulties, the tail-cuff" technique involves andardized heating. The blood-flow in the tail e es thereby increases about hundredfold (Johansen 1962) Heating can obviously only make an adequate blood-flow in the tail more or less probable but it cannot ensure such conditions (Sobin 1946 Bunag et al 1971) Furthermore the procedure might cause overheating discomfort and possibly itself induce rise in blood pressure (Proskauer et al. 1945)

In order to avoid some of the difficulties associated with heating, it would be advantageous to be able to establish set of criteria for adequate measuring conditions based on observations on blood-flow in the tail made simultaneously with the blood pressure recording.

The aims of the present study have been to

(*a*) determine the relation between body temperature and pulse volume in the tail (an indirect measure f blood-flow Hellström 1975)

(*b*) determine how the error in the indirect blood pressure reading varies as a function of pulse volume in the tail

METHODS

The total of 47 Sprague-Dawley albino rats, 150–620 g, were used a acute experiment relating blood pressure to

...als tested underwent a period where body ...erature exceeded 38° in spite of having a vaso-...traction with a pulse volume less than 20% of ...mum. It is also to be noted that large amplitude ...tions were recorded at normal and sub-normal ...y temperatures at the end of the experiment. A ...plex relation between body temperature and ...e volume was a typical finding.

...ability of indirect blood pressure measure-...nts. The direct and indirect blood pressure were ...pared at various pulse volume amplitudes in ... under anesthesia. Fig. 2 shows the relation ...$I/D \times 100\%$ between the direct systolic (D) ...indirect (I) pressures as a function of relative ...e volume measured immediately before the in-...on of the cuff. An exponential function was ...pted to the experimental values by a non-linear ...ession technique. It is seen that below about ...% of maximum of pulse volume, the indirect ...sures are variable and usually much below the ...ct pressures. Above approximately 25% the ...relation between indirect and direct measures ...ws no further improvement. Regression lines ...e calculated for all values above 20% for each ...mal. Mean slope of these lines was close to zero ...0.01 S.D =0.078 N=7). In most cases a static ...or remained which amounted maximally to 20% ...he intra-vasal pressure values. As the same cuff ...s used on all animals irrespective of animal size ...h an error is to be expected (see e.g. Fregly ...3). The relation between this steady state error ...en pulse volume was large) and the body weight ...l sex of the animal was determined for 36 ani-...ls. The large animals (males) had a higher blood ...ssure in the tail (indirect $\bar{x}$=5.3% S.E.=2.2) ...n in femoral artery. Small animals (most of them ...nales) had a 8.6% (S.E.=3.3) lower indirect than ...ect pressure.

CONCLUSIONS

The present results show that:

1. The pulse volume (blood-flow) is not a simple function of body temperature.

2. The animal has to be heated to give a pulse volume in the tail of at least 20–25% of the maximum in order to allow adequate indirect blood pressure readings.

3. An error remained (with 20 mm cuff) in spite of heating. The indirect pressure in full-grown male rats was 5–10% higher than the direct pressures whereas it was 5–10% lower in females.

4. Pulse volume recorded from the surface of the tail is more reliable than body temperature or ambient temperature for standardization of measuring conditions when utilizing the "tail-cuff" technique.

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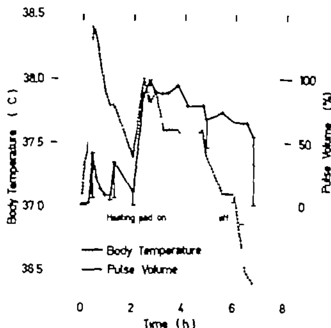


Fig 1 Typical example of relative pulse volume in the tail and rectal temperature various times after initiation of heating and during subsequent cooling of a non-anesthetized rat Vertical bars indicate range of pulse volume values during a 5-min observation period

arterial pulse volume and pulse volume to body temperature

Recording of pulse volume and blood pressure The blood-flow in the tail was indirectly assessed by surface recorded arterial volume pulsations (Hellström 1975) A rubber balloon was taped to the ventral side of the tail and connected to an Elema 510C transducer whose signals were amplified and recorded on an Elema T34 recorder Blood-flow was occluded at the base of the tail by an inflatable cuff 12×70 mm lined with latex rubber The pressure in the cuff was raised rapidly to suprasystolic levels and then gradually released The cuff pressure measured by a modified commercially available system (Technilab) was monitored on the recorder and on a standard sphygmo manometer The i.a blood pressure was obtained by connecting the iliac artery to a Statham P23 DC transducer with a PE 50 or PE 60 tube

The animals were heated either by a temperature-controlled heating-pad (Anitemp Jacoby Sweden) or by a conventional light bulb Rectal temperature was continuously monitored on a galvanometric thermometer (Elektrolaboratoriet Copenhagen)

Procedure For comparison of direct and indirect blood pressures the animals were kept in pentobarbital anesthesia and heated to produce various amplitudes of pulse volume (blood-flow) Simultaneous readings of direct and indirect pressure were made at widely different values of pulse volume The maximum pulse amplitude was obtained after considerable overheating of the animals For comparison between body temperature and tail pulse volume non-anesthetized animals were used The rats were adapted to the measuring situation during a 5-day training period During the experimental sessions the rat was initially anesthetized with ether and placed on the heating-pad in the wiremesh tube used for restraint. Rectal temperature and pulse volume were continuously recorded on the non-anesthetized animal during heating and subsequent cooling over a total period of 4-8 hours

RESULTS

Relations between body temperature and pulse volume in the tail In the measurements on anesthetized animals the relation between body temperature and pulse volume was variable Radiant warming was more efficient in increasing pulse volume (in spite of low body temperature) than the usage of the heating pad These observations may however not be relevant for blood pressure measurements with the tail-cuff technique since measurements are regularly made on non-anesthetized animals

In the experiments on non-anesthetized rats the body temperature and pulse volume could be more adequately compared Fig. 1 shows a typical example of rectal temperature and relative pulse volume as a function of time after start of the experiment It is seen that the body temperature was initially 37° and that it could be raised to 38° without being accompanied by any appreciable increase of pulse volume When the vessel dilated body temperature usually dropped almost to normal and then increased again A long period of variable pulse volume (vertical bars) is observed in Fig 1 before steady maximum dilatation occurs Seven of the

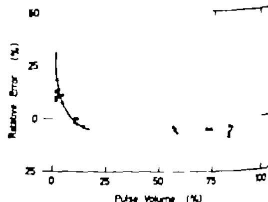


Fig 2 Relative difference between intra-arterial and tail cuff pressure in an anesthetized rat as a function of degree of pulse volume (blood-flow) in the tail *Ordinate* (Direct Indirect)/Direct × 100%. *Abscissa* Pulse amplitude in percent of maximum obtainable value Dots indicate observed values. Adapted exponential function (continuous line): $y=a+be^{-cx}$ *a* indicates steady state error (6%) and $c=0.19$

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ta-adrenergic dilator interaction with the constrictor ,ponse in resistance vessels of skeletal muscle ring hemorrhage

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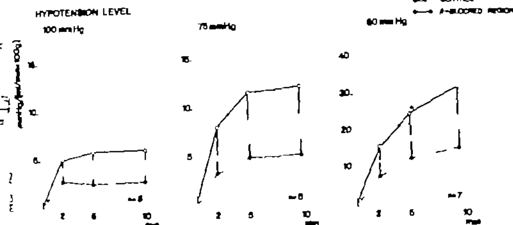
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HILLMAN J & LUNDVALL J Beta-adrenergic dilator interaction with the constrictor response in resistance vessels of skeletal muscle during hemorrhage. Acta Physiol Scand 1980, 108 77–83 Received 14 May 1979 ISSN 0001-6772. Department of Physiology and Biophysics, University of Lund Sweden.

A marked β-adrenergic dilator interaction with the vasoconstrictor response in skeletal muscle during hemorrhage is described The dilator influence corresponded to some 40 % of the constrictor response both at mild nd moderate s well as at large bleeding. In absolute resistance units the β-adrenergic dilator influence averaged no less than 14 mmHg/(ml min 100 g) at large bleeding (hemorrhagic hypotension of 50 mmHg). Comparison of the hemorrhage induced resistance effects in the autoperfused innervated muscle in the autoperfused denervated muscle and in the innervated muscle cross-circulated from donor animal showed that the β-adrenergic dilator influence more or less completely was caused by blood-borne catecholamines in all likelihood by adrenaline which is known to be secreted in large amounts during hemorrhage. The described β-adrenergic dilator mechanism may serve to maintain nutritional blood flow by counter acting the constrictor response It deserves consideration also from the point of view that it obviously has to be taken into account for proper evaluation of other vascular control mechanisms brought into action in hemorrhage

ch effort has been devoted to the problem of ulation during hemorrhage of peripheral vascular stance in hemodynamically important vascular s. Skeletal muscle with its large tissue ma s, one of these main targets for vasomotor re- nses in the body reflex adjustment of blood ssure during bleeding (see e g Chien 1967) The rease of muscle resistance during hemorrhage been attributed to act ve vasoconstriction luced by the ympathetic vasomotor fibres and vasoactiv hormone e g the catecholamines giotensin and vasopressin, and further to pas- e factors such as elastic recoil of the vascular lls esponse to the decreased transmural pres- re and rheological phenomena

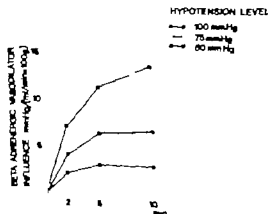
The vasocon trictor response in muscle dur g morrhage has been reported to be modified by al chemical d lator factors accumulating as result of the reduced blood flow especially during prolonged periods of more severe bleeding (e.g Mellander & Lewis 1963) Apart from this little attention has been paid to the possibility that antagonistic dilator interaction with the vasoconstrictor influence may be of importance in determining the net resistance effect despite the fact that such possible interaction might have important functional implications primarily with regard to tissue nutri tion. A previous study performed on cat skeletal muscle (Lundvall & Hillman 1978) showed how ever that hemorrhage may be associated with quite a pronounced β-adrenergic nhibitory influence on vascular tone i the resistance vessels. In the present investigation this inhibitory resistance re sponse was studied n more detail in order to evaluate its quantitative importance during graded hemorrhage and to reveal the mechanisms responsible for the reaction.



1 Increase of muscle vascular resistance during graded hemorrhage in the autoperfused innervated regions with [intact] and [wi]th blocked β-adrenoceptors. [...] statistical difference at $P<0.05$ and ** = $P<0.01$

[me]an values ± S E) refer to the autoperfused [inne]rvated, autoperfused denervated and cross-[perfu]sated innervated muscle regions with intact β-[adre]noceptors ("intact" region) and with blocked β-[adre]noceptors (β-blocked region) respectively [It c]an be seen that resting vascular resistance aver[age]d 16-17 mmHg/(ml × min × 100 g) in the in[ner]vated intact region and 13 mmHg/(ml × [...]) in the denervated intact region. These [resi]stance values are normal for cat skeletal muscle [(se]e Lundvall 197[?]). The values for vascular resist[an]ce in the β-blocked muscle region did not differ [sig]nificantly from those in the intact region.

[F]ig. 1 summarizes the observed resistance [ch]anges in the autoperfused, innervated muscle in [ter]ms of the increase of regional resistance (mmHg/ [(ml]× min 100 g)) [a]bove control level after 5 [and] 10 min at each hypotension level (mean values [±] S E note the different scales along the ordinate) [In] the muscle region with intact β-adrenoceptors [(b]roken line) a[s] well as in the β-blocked muscle [reg]ion (solid line) the resistance response was [gra]ded in relation to the degree of hypotension, but [at] each hypotension level the [i]ncrease of resistance [aft]er β-blockade showed a clear tendency to be [mo]re pronounced than [in] the muscle region with [int]act β-adrenoceptors (cf. significance values [giv]en in the diagrams) This discrepancy implies [th]at hemorrhage was associated with [a] clear-cut [β-]adrenergic dilator influence [i]n the resistance [ves]sel of the autoperfused, normally innervated [sk]eletal muscle vascular bed I[n] the diagram of Fig. 2 this β-dilator effect is expressed as the dif ference between the resistance increase in the β-blocked and in the intact muscle region. It can be seen that the dilator influence was graded in relation to the degree of hemorrhage and after 10 min the β-dilator influence averaged 3.[.], 6 9 and 13 9 mmHg/(ml × min × 100 g [)] at the hypotension levels [o]f 100 75 and 50 mmHg. During large bleeding (50 mmHg) the dilator effect thus indeed was pronounced. When expressed in relative terms however the β-adrenergic dilator influence was quite significant also at mild (100 mmHg) and moderate (75 mmHg) hemorrhage as indicated by the



F[i]g 2 Calculated β-adrenergic vasodilator influence in the autoperfused innervated muscle during graded hemorrhage

Table 1 *Mean values ± S E in the control state before hemorrhage for arterial blood pressure, regional blood flow and regional vascular resistance in the innervated autoperfused lower leg muscle region, in the denervated autoperfused muscle (n=5) and in the cross-circulated muscle region (n=6)*

Paired and concomitant observations were obtained on the left lower leg muscles with intact and on the right muscles with blocked β adrenoceptors

	Innervated control region (n=8)	Denervated region (n=5)	Cross-circulated region	
			Recipient	Donor
Intact region				
Arterial pressure mmHg	133±3	1 4±3	141±6	13.+1
Regional blood flow ml/min×100 g	8.5±0 4	9 4±0 4	7 9±0 7	
Regional resistance mmHg/(ml/min×100 g)	16 9±1 1	12.8±0 4	16 9±1.8	
β-Blocked region				
Arterial pressure mmHg	136±3	125±3	141±6	13._1
Regional blood flow ml/min×100 g	8 0±0.5	8 9±0.5	7.2±0.5	
Regional resistance mmHg/(ml/min×100 g)	17 4±1 6	13.5±0 4	17 1±1.2	

METHODS

The study was performed on 19 cats (? 6–3 8 kg) anesthetized i v with chloralose (50 mg/kg b wt) and urethane (100 mg/kg b wt) after induction with ether

Observations were made on two identical muscle regions viz the right and left lower leg muscles which were isolated so that on each side the popliteal artery and vein formed the sole vascular connections with the main part of the body The muscles were wrapped in gauze soaked in saline and covered by a plastic sheat to preserve a moist environment. Muscle temperature was maintained normal using a heating-lamp After heparinization (750 IE/kg b wt) venous outflow from each muscle region was diverted from the popliteal vein to the right external jugular vein An optical drop-recorder was inserted bilaterally in the venous shunt for continuous recording of the blood flow A short shunt circuit connected the femoral and popliteal artery on each side and was used for regional administration on propranolol in order to block the β-adrenoceptors The right brachial artery was cannulated and connected to a siliconized graduated pressure bottle the pressure of which could be adjusted to desired levels Arterial blood pressure was monitored from a T-tube in the cannula in the brachial artery Venous outflow pressure determined by the height of the orifice of the venous outflow tubing was maintained constant at 5 mmHg on both sides

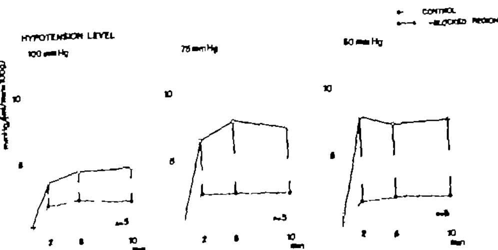
Changes of vascular resistance in response to hemorrhage were studied on the autoperfused innervated muscles on the autoperfused denervated muscles and on the cross-circulated innervated muscle region The sciatic nerve which contains virtually all sympathetic fibres to the lower leg muscles was left intact on both sides in 8 animals and severed bilaterally in 5 animals both groups of animals being autoperfused In 6 animal with intact sciatic nerves the lower leg muscles were supplied with blood from a donor cat by connecting with siliconized polyethylene tubings the femoral arteries of the donor to the popliteal arteries of the right and left lower leg muscle preparations of the recipient The outflow of blood the cross-circulated muscle region was passed the optical drop-recorders and returned to the donor via the right external jugular vein

Hemorrhagic hypotension was produced by rapid ing into the pressure bottle Stepwise graded bleed the three hypotension levels of 100 mmHg 75 mmHg 50 mmHg was performed At each hypotension level arterial pressure was maintained constant for 10 min that the total period of hemorrhage amounted to 30 After reinfusion of the shed blood the animals rest another 30 min and were then subjected to a se identical period of stepwise bleeding The volume of that had to be withdrawn to achieve the arterial hyp sion levels of 100 75 and 50 mmHg was quite similar autoperfusion and crosscirculation experiments, on average it amounted to 7 15 and ml/kg b wt During sequences of stepwise bleeding, the right lower leg preparation was subjected to regional blockade of β-adrenoceptors by propranolol whereas the β-adrenoceptors of the muscle preparation on the left leg intact. The β-blocking agent was administered arterially into the arterial shunt circuit in a dose of muscle Concomitantly venous outflow of blood was pled and discarded to avoid systemic effects of the blocking agent The discarded blood was substituted by administration of the same volume of dextran

In Results section each presented observation reached by calculating the mean of the two responses obtained in the separate periods of bleeding the same animal Spread of data is expressed as S E significance test were performed according to Student's t test

RESULTS

Table 1 gives data on arterial blood pressure regional blood flow and regional vascular resistance in the control state before bleeding The



5 Increase of regional vascular resistance during graded hemorrhage in the autoperfused denervated muscle with t and th blocked β-adrenoceptors =statistical difference at $P<0.05$ and $=P<0.01$

s that bleeding is associated with a marked renergic dilator influence in the resistance el of skeletal muscle In absolute res stance the dilator influence was found to be related e degree of hemorrhage and at large bleeding effect reached no les than 14 mmHg/(ml× 100 g) When calculated in relati e terms dilator influence was marked at all levels of ding Thus it was deduced to correspond to e 40% of the constrictor response both at light moderate as well as at large bleeding (Fig 3). functional significance of such an inhibitory t on vascular tone remains to be clarified perhaps primarily serve as mechanism for tenance of nutritional blood flow during the orrhage induced vasoconstriction However described dilator mechanism deserves con ation also from the point of view that it has to ken into account for proper evaluation of other ular control mechanisms brought into action in orrhage

he three different series of experiments per ed on the autoperfused nervated muscle on autoperfused denervated muscle and on the s-circulated innervated muscle respectively le it clear that the observed β-adrenergic dila influence mor or less completely was caused blood-borne catecholamines, in all likelihood ly by adrenaline which is known to be released arge amount during hemorrhage (e g. Watt Hall & Hodge 1971 Jakschik et al. 1974) analogous adrenaline effect on muscle resist ance was reported by Chalmers et al (1966) in rabbit subjected to se ere hypoxia. The fact that the β-adrenergic dilator response was significantly smaller in the denervated than in the innervated autoperfused muscle at marked hypotension (50 mmHg) may not necessarily contend with this conclusion. It is known for example that the magnitude of a vascular effect often depends on the pre vailing level of vascular tone (e g. Myers & Honig 1969 Lundvall 1972), and this level differed mark edly in the mentioned two preparations The lack of any significant direct, nerve mediated β-adrenergic decrease of muscle vascular resistance dur ing the hemorrhage induced reflex increase of sympathetic nerve discharge as demonstrated by the cross-circulation experiments is in accordance with a previous study using artificial electrical activation of the sympathetic nerves to skeletal muscle (Lundvall & Järhult 1976). These stimulation experiments failed to reveal any neurogenic β-adrenergic dilator effect on total muscle ascular resistance although there were a quite marked microvascular dilator influence. The blood-borne catecholamine induced β-adrenergic inhibition of vascular tone during bleed ng which entirely must represent a regional effect in the muscle vascula bed, probably reflect direct interaction with specific receptors of the vascular smooth muscle cells Indirect effects secondary to adrenergic alterations of the skeletal muscle metabolism can not be ex cluded however Possible interaction with pre synaptic β-adrenoceptors facilitating transmittor

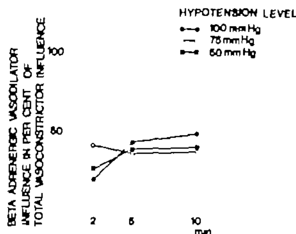


Fig 3 β-Adrenergic vasodilator influence in the autoperfused innervated muscle during graded hemorrhage in relation to the concomitant vasoconstrictor influence

data in Fig 3 The extent to which the β-adrenergic dilator influence counteracted the simultaneous constrictor influence has here been deduced in rough terms by dividing the β-dilator effect (Fig. ?) with the total resistance increase observed in the β-blocked muscle region (Fig 1) The data suggest that the constrictor response was reduced by some 40% as a result of the β adrenergic dilatation at all three levels of bleeding

It would seem likely that the observed β-adrenergic dilator influence in the autoperfused innervated muscle during bleeding was exerted by blood-borne catecholamines released from the adrenal medulla but it may also be related to a β adrenergic dilator component of the vascular response evoked by the adrenergic vasomotor fibres (cf Discussion) The latter possibility however seems refuted by the results obtained in experiments in which the innervated lower legs were cross-circulated from a donor animal (see Methods) In these experiments the concomitant resistance responses during bleeding of the recipient to 75 mmHg were followed in the left limb with intact β adrenoceptors and in the β-blocked right limb The results are presented in Fig 4 which shows that the evoked increase of resistance was very similar in the intact and β blocked muscle region

The β-adrenergic dilator influence observed in the autoperfused innervated muscle (Figs 1 and ?) thus seems attributed to the action of blood borne catecholamines In an attempt to further elucidate such a humoral dilator effect the resistance function was studied in the autoperfused denervated muscle region during graded bleeding to the [illegible]tand ardized hypotension levels of 100 75 and 50 [illegible] The collected results from these experiments [illegible] shown in Fig 5 (mean values ± S E.) In the [illegible] muscle region hemorrhage evoked an increa[illegible] resistance which was fairly small and of [illegible] magnitude at the different hypotension levels [illegible] mmHg/(ml×min ×100 g ⁻¹)) Also in the β-bl[illegible] muscle region the response was roughly sim[illegible] the three levels of hypotension but resistance [illegible] clearly more raised than in the intact muscle, [illegible] 5–8 mmHg/(ml×min⁻¹×100 g⁻¹) These exp[illegible]ments thus directly reveal quite a marked hum[illegible] β-adrenergic dilator influence during hemorrh[illegible] The magnitude of this dilator effect in the dene[illegible]ted muscle as reached by subtracting the resist[illegible] response in the intact region from that observ[illegible] the β-blocked region amounted to some 3 [illegible] (ml×min⁻¹×100 g⁻¹) at mild hemorrhage a[illegible] about 5 mmHg/(ml×min⁻¹×100 g⁻¹) at mod[illegible] and large hemorrhage The dilator effects at [illegible] and moderate bleeding are of similar magnit[illegible] to those presented in Fig. ? for the experimen[illegible] the autoperfused innervated muscle whereas [illegible] β-adrenergic dilator effect at large hemorrhage [illegible] mmHg) was much more pronounced in the in[illegible]vated than in the denervated muscle (see Dis[illegible]sion)

DISCUSSION

The present study which confirms and extends previous observations (Lundvall & Hillman 19[illegible]

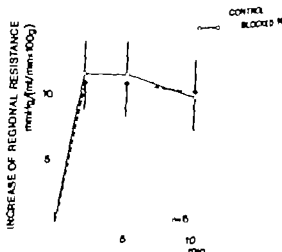


Fig 4 Increase of muscle vascular resistance during hemorrhagic hypotension (75 mmHg) in the cross-circulated lower leg muscle with intact and with blocked adrenoceptors

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release from the adrenergic nerve endings during nerve discharge (cf Langer 1977) also deserves consideration especially since this mechanism may be activated by blood-borne catecholamines (e g Stjärne & Brundin 1976) It can be deduced that blockade of any such facilitation in fact would lead to an underestimation of the magnitude of the post synaptic effector cell located β adrenergic inhibitory effect on vascular tone

As mentioned previously skeletal muscle is one of the tissues that contribute in an important way to the regulation of overall peripheral resistance during hypovolaemia (for ref see Chien 1967) Many studies indicate that the evoked vasoconstriction mainly is due to increased discharge in the sympathetic vasoconstrictor fibres (e g Öberg 1964 Lundgren Lundvall & Mellander 1964 Haddy Scott & Molnar 1965 Bond Manley & Green 1967) The blood-borne catecholamines have been proposed to contribute more or less markedly to the constrictor response (Bond Manley & Green 1967 Hall Schwinghamar & Lalone 1976) and vasoactive amounts of vasopressin (e g Rocha E Silva & Rosenberg 1969 Cousineau Gagnon & Sirois 1973) and angiotension (cf Gutmann et al 1973) may be released as well Experimental evidence clearly indicate that species differences can be quite marked and further that the relative role of different vasoconstrictor stimuli varies with the type of bleeding performed (e g rapid or slow exsanguination) and with other methodological factors such as whether pressure bottle was used or not The importance of the last mentioned factor for example may be demonstrated by the findings that the alfa-adrenergic constriction as well as the β adrenergic dilatation increased significantly with time during large bleeding (Figs 1 and 2) whereas both these responses soon reached a steady state in *similar experiments without pressure* bottle (unpublished data) This difference may be related to the greater stress the animal encounters in the pressure bottle experiments insofar that they can not benefit to full extent from the cardiovascular compensatory mechanism brought into play As stated above proper evaluation of the nature and magnitude of vasoconstrictor mechanisms also requires that possible and quantitatively important antagonistic dilator actions can be taken into account The marked β adrenergic dilator influence revealed *in this* investigation is obviously one such factor and the necessity to account for it may be demonstrated by the results obtained in the [illegible] vated autoperfused muscle preparation (Fi[illegible] The resistance effects observed in the [illegible] preparation with intact β-adrenoceptors [illegible] impression of a weak humoral vasoconstr[illegible] fluence Yet the observations in the β-b[illegible] muscle region reveal the presence of a pro[illegible] nounced hormonal vasoconstriction during [illegible] ing This vasoconstrictor influence seems caused by activation of alfa-adrenoceptors [illegible] can be abolished after administration of dibe[illegible] (unpublished observations)

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Physiol Scand 1980 108 85–90

ıe course of adaptation to low intensity ning in sedentary men dissociation of ıtral and local effects

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The oxygen transporting capacity and the metabolic capacity of the vastus lateralis muscle ere followed in parallel in 9 sedentary overweighted men during low intensity training program Measurements were made at 0 3 6 9 1 and 30 weeks Maximal oxygen uptake increased in an approximately linear fashion during the first 1 week (11%), but decreased little (3%) during the following 18 weeks Mean body weight decreased 8% (7 4 kg) during the training The distribution of muscle fibre types, including the subgroups of type II fibres, did not change Muscle enzyme activities remained essentially unchanged during the training It wa concluded that central" och "local" adaptation need not occur in parallel, and that the leg xygen utilization capacity probably does not limit the whole body' maximal oxygen uptake

Key ords Low intensity training, muscle metabolism oxygen uptake time course

intensity training of a kind that can be carried mainly unsupervised by previously sedentary ple has been show to elicit physiological adaptions at the ystemic as well a at the muscle ell el (e g Kilbom 1971 Pollock 1973 Örlander et 1977) In previous investigation (Örlander et al 7), e found that the increases in maximal gen uptake ($\dot{V}_{O_2}$ max) and muscle metabolic tential ere asynchronous and probably not ectly related Furthermore it appeared that the ne course of the training response might be differ t for different metabolic pathways in muscle The present study was undertaken as an attempt larify these point by following the time course changes in $\dot{V}_{O_2}$ max and skeletal muscle enzymes in sedentary overweighted men during a low ensity training program

SUBJECTS AND METHODS

Subjects 9 clinically healthy sedentary and overweighted men volunteered to participate thi study An oral consent was obtained from each subject after he had been informed of the procedure and possible risks of the experiment They were all untrained had white-collar jobs and had never been engaged in any regular physical training Pertinent anthropometrical data (mean, S D range) for the whole group were age 33±4 (22 35) years, height 183 7 (174–193) cm and weight 97 6 9 4 (84 7–114 0) kg Five ere smokers and 4 nonsmokers, but 3 of the smokers stopped smoking during the training period

T aining regimen The first part of the training period lasted 12 weeks. During this period of time the subjects trained on their own about 4 km 5 times week and had

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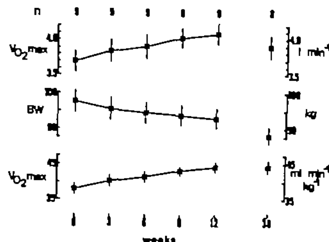


Fig 1 Maximal oxygen uptake ($\dot{V}_{O_2}$ max) and body weight (BW) at various times during the training (means ± S E.) The number of subjects at each test occasion is given at the top All means at weeks 3–30 are significantly different from the 0 week means (P<0 01–0 001)

weekly contact with the investigators Training intensity was decided from the pre-training tests (see below) and aiming at an average oxygen uptake ($\dot{V}_{O_2}$) during the training workouts of 60–80 per cent of maximum During the first 6 weeks the training consisted of "fast walking" During the following 6 weeks 1 or of the walking work outs included some slow jogging (for definition of d f ferent exercise speeds and corresponding average load on oxygen transport see Ekblom et al (1979)) The subjec tive rate of perceived exertion (RPE) corresponding to 80 per cent of $\dot{V}_{O_2}$ max was determined according to the Borg scale (1962) during the exercise tests The subjects were asked not to exceed this RPE during train ng

From 12 weeks on the subjects still trained on their own but had no contact with the investigators until the final test after 30 weeks In this training period of 18 weeks the subjects were asked to continue with the f st walking–slow jogging program The average attendance of training during this period was 3 times a week One subject could not participate in the 30-week follow up since he had moved abroad

Exercise te ts Preliminary tests were performed to familiarize the subjects to the test methods used and to obtain preliminary data on $\dot{V}$ max and maximal work loads Before the training tarted at each 3rd week up to the 12th week and after 30 week the same test procedure was applied Each subject was we ghed early in the morn ing after a light meal With ECG electrodes attached he performed 1 or submaximal 6 min work periods on a mechanically braked Monark bicycle ergometer and af ter a short warming up -period an exhaust ve run (work time 4–6 min) on a motordriven treadmill On another test day before the training started and at the 6th and 12th week s test the subjects performed 6 min exercise period at to 4 different speeds which were subjecti ely deter mined namely normal walk fast walk slow jog and normal jog (for details see Ekblom et al 1979) The latter tests were used for the prescription of the training speed Since this paper deals with the central" and local" adap-

tation to a low intensity training program the da this part of the study will be published elsewhere

On all work loads $\dot{V}_{O_2}$ was determined between t and 6th min of the submaximal work and at lest urements were made towards the end of the ma using the Douglas bag-Haldane technique Her (HR) was calculated from the ECG recorded d during submaximal and continuously at the end of mal exercises

The subjects were asked not to change the dail habits except excluding alcohol and between m snacks However it cannot be excluded that so them stopped smoking some of the subjects also ch the quality and quantity of the calory intake

Mu cle analyses Biopsies were taken from the v lateralis muscle with a needle technique (Bergström alternating between the right and the left leg The s mens were used for enzyme assays and for histoch try

For enzyme activity determinati ons homogenates prepared and assays performed as described prev (Örlander et al 1977) except that assays ere ma The investigated enzymes chosen to represent the pathways in energy metabolism were phosph tokinase (PFK E C. 7 1 11) lactate dehydrog (LDH E C 1 1 1 27) 3-hydroxyacyl-CoA dehydrog (HAD E C.1 1 1.35) citrate ynthase (CS E C 4 and cytochrome oxidase (cytox E.C 1 9.3 1) PFK a ty was estimated according to Shonk & Boyer (LDH and HAD by the method of Bass et al (1969), C described by Srere (1969) and cytox according to W et al (1969) Muscle protein wa e timated by the me of Lowry et al (1951)

For histochemi try a muscle specimen was im oriented mounted in Cryoform™ frozen in isop cooled by liquid nitrogen and stored at 80°C analyzed Transverse section (10 μm) were cut cryotome and stained for myofibrillar ATPase aft kal ne (pH 10.3) or acid (pH 4.3–4 6) preincub (Padykula & Herman 1955 Guth & Samaha 1969 Br & Kaiser 1970) Fibres were cla ified as type I (twitch) or type II (fa t twitch) (Engel 1962) In biopsies type II fibres were subclas ified into II A and IIC (Brooke & Kaiser 1970 Dubowitz & Br 1973) An average of 154 fibres were counted per bi The variability in percent type I fibres between bi specimens taken from the same subject wa (mean ± S D calculated from 86 pairs)

Statistical evaluation of changes and inter-group d ences wa made with the paired and independent t te respectively The level of significance was selected P<0 05

RESULTS

$\dot{V}$ max increased in an approximately linear fash during the first 1 weeks of training reaching value of 10 8% above the pre training le (P<0 001) (Fig 1) A significant (4 1% P<0 increase was observed already at 3 weeks Beca the mean body weight decreased there was a gr

I Distribution of mus le fibre types in astus laterali at various tim during the t aining

are mean percentages ±S E. The number of subjects is given within brackets. *P* values refer to the difference compared th the 0 eek value

	Week of training					
	0	3	6	9	1	30
	3 1±6 8 (9)	29.5±4 3 (9) >0 50	3_1±6 6 (7) >0 70	32.3_7.0 (5) >0.50	27.4±4 0 (7) >0 30	26.9±2.3 (6) >0.90
	33 7 7 3 (7)		–		30 4±4 9 (5) >0 30	30 8±2.6 (5) >0.40
	44 3±6 1 (7)				4_4±6 0 (5) >0 60	41 7_4 2 (5) >0.30
	21 7±7.8 (7)				26 9±4 7 (5) >0 60	27.2±_8 (5) >0 70
	0 3±0 (7)				0 4±0 2 (5) >0.90	0.3±0.3 (5) >0 90

elative improvement in $\dot{V}_{O_2}$ max per kg body ght. At 1 weeks a 16.6% increase ($P<0.001$) seen (Fig. 1). In the 30 week test $\dot{V}_{O_2}$ max in remaining 8 subjects had decreased 3 0% 0 01) from their 1 week mean value but was significantly higher than before training (8 0% 0 01) Because body weight continued to de ase $\dot{V}_{O_2}$ max per kg body weight remained un- nged (Fig 1). Those subjects with the lowest -training $\dot{V}_{O_2}$ max tended to show the greatest ative increases at 1 weeks There was however significant correlation ($=-0$ 47 $P>0$ 20).

The mean percentage of type I fibres in the vastus eralis was 32 1% before training, and no signifi- t change was seen during the training. Neither there any change n the distribution of type II groups (Table 1). For technical reasons, biop- could not be taken in some instances, and some opsies could not be analyzed. However virtually ntical result were obtained when only subjects o could be analyzed at all time points were con- dered Furthermore statistical evaluation was sed on intraindividual comparisons only The me reasoning applies to the enzyme activity data ell

As shown in Fig. _ muscle enzyme activities ere essentially unchanged during the course of the udy At 6 weeks, LDH was lowered ($P<0.05$) d the same was true for CS at 12 weeks ($P<0.01$). tendency ($P<0.10$) towards increased cytox ac ities was evident at 12 weeks. No significant hanges in muscle protein concentration were ob- rved

Correlations between the studied variables wer computed, and the following significant relation- ships were obtained % type I vs. $\dot{V}_{O_2}$ max at 6 weeks ($r=0$ 76) % type I vs cytox at 3 weeks ($r=0$ 68) % type I vs PFK at 12 weeks ($r=0.84$) CS vs HAD at 3 9 12 and 30 weeks ($r=0.82$–0 98). No significant correlation between % type I fibres and the increase in $\dot{V}_{O_2}$ max was observed

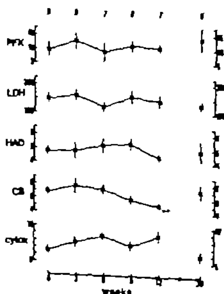


Fig 2 Enzyme activities in vastus lateralis at various times during the training (means ± S E.). Numbers of subjects are indicated at the top Activities are given as µmol min (g wet wt)⁻ for phosphofructokinase (PFK) lactate dehydrogenase (LDH), 3-hydroxyacyl-CoA dehydrogenase (HAD) and citrate synthase (CS), and as µmol O_2 min (g wet wt)⁻¹ for cytochrome oxidase (cytox). and ** denote $P<0.05$ and $P<0.01$ respectively when compared with the 0 week value.

Pre training data for smokers ($n=5$) and nonsmokers ($n=4$) were not significantly different except for a higher ($P<0.01$) percentage of type II A fibres in the smokers Non-significantly lower mean values for $\dot{V}_{O_2}$ max and % type I fibres were seen in the smokers (3.51 vs 3.91 l×min⁻¹ and 27.1 vs 38.4% respectively) Since 3 of the smokers quit smoking during the study no meaningful comparison of the training effects in smokers and nonsmokers was possible

DISCUSSION

In the present study the capacity of the oxygen transport system and the metabolic capacity of the vastus lateralis muscle were followed in parallel during a low intensity training program The maximal oxygen uptake increased considerably while the skeletal muscle characteristics remained essentially unchanged (Fig 1 and 2 Table 1) This indicates that the type intensity and duration of training is of great importance for whether "central" and local adaptation will occur in parallel or not

The increase in maximal aerobic power in the present study was similar to that in other low intensity training studies (Kilbom 1971 Örlander et al 1977) An initial increase rate of about one percent per week appears to be typical for this kind of training It should be noted that due to the simultaneous weight reduction there was in the present subjects a substantial increase in $\dot{V}_{O_2}$ max per kg body weight

Evidently the increased metabolic demands due to the employed training program were within the capacity of the vastus lateralis muscle (Fig.) The decreased LDH activity at 6 weeks may be a chance finding Such a decrease is however regularly observed during extensive endurance training (Holloszy & Booth 1976 Sjödin 1976) CS showed a decreasing trend from week 3 and was significantly lowered at 12 weeks The reason for this is not clear One could speculate that other factors than the training such as the changed dietary habits might play a role It may be noted that although the well-established correlation between CS and HAD (Staudte & Pette 1972 Bylund et al 1976, Örlander et al 1977) was found also in the present study no significant changes in HAD activity were observed

It seems clear that the adaptive response in skeletal muscle is related to the work performed during training. Exercise of low intensity or short daily duration has been shown not to influence metabolic capacity of rat hindleg muscles (He. Wainio 1956 Holloszy 1967 Saubert et al Benzi et al 1975 Åström, personal communication) With an increased training intensity or duration a progressive increase in mu. enzyme activities towards a new steady state takes place (Kraus et al 1969 Barnard & 1971 Guy & Snow 1977 Henriksson & Re 1977 Baldwin et al 1977) It appears that the intensity is of major importance when the duration relatively short (Benzi et al 1975) At high intensity the duration per workout is the determinant for the training response (Fitts 1975 Dohm et al 1977) Ultimately a threshold is reached beyond which further prolongation workout is without effect on muscle metab (Terjung 1976 Vihko et al 1978)

The lack of effect on muscle metabolism n present study is in contrast to our previous fi (Örlander et al 1977) The reason for this is p ably the differences in the employed training grams About one third of the training session the previous study consisted of calisthenics, w aim to increase muscle power whereas n training was carried out in the present s Bylund et al (1977) using a training program volving leg strength training found that the incr in oxidative capacity was largely confined to type II fibres Since endurance running (and p ably also walking) is performed mainly with the of type I fibres (Costill et al 1973) it is reasonable to conclude that low intensity exercise within the capacity of this fibre population A h work load involving also the type II fibres then be required to elicit an adaptive response.

In conclusion the present results show that maximal aerobic power can increase consider without a corresponding change in muscular ox tive capacity Thus leg muscle oxygen utiliz capacity does not seem to limit the whole bo $\dot{V}_{O_2}$ max This conclusion is in accordance our previous observations on low intensity tra (Örlander et al 1977) and with the results o cent studies on detraining showing a clear dis tion between changes in $\dot{V}_{O_2}$ max and muscle zyme activities (Henriksson & Reitman 1 Houston et al 1979) Further support for this clusion comes from the numerous studies when arterial oxygen content has been manipulated ing maximal work (e g Ekblom et al 1975 1!

...rom experiments on maximal work involving ...rent amounts of the body's muscle mass (Bergh ...) 1976). As Clausen (1977) has pointed out, it ...ms clear that $\dot{V}_{O_2}$ max during work with large ...cle groups is limited by oxygen supply. It is, ...ever, possible that an elevated oxidative capa... ...in leg muscles other than the vastus lateralis ...t have made some contribution to the increase ...$_{O_2}$ max. It has been shown that the gastrocne... and soleus are considerably more involved ...ing level running, whereas the vastus lateralis is ...t equally active during uphill or downhill run... ...(Costill et al. 1974). To our knowledge, no data ...available as to the relative involvement of dif... ...nt leg muscles in the kind of walking and jog... ...activities employed in the present study.

...nvestigation has been supported financially by the ...arch Council of the S...edish Sports Federation (grant ...6). W... are indebted to Mrs Carina Eriksson for sec... ...ial assistance.

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mparison of mucus collecting methods fish olfaction

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A new method for collecting non-contaminated skin mucus from fishes is introduced The method is compared with customary method by gel filtration, analysis of free amino acids, and determination of dry weight Skin mucus collected by the "customary" method is shown to be contaminated by intestinal juice The ole of skin mucus as source of fish odorant affecting fish behaviour is discussed in the light of the above findings.

Key ords Amino acids, fish odour mucus, olfaction salmonids fish behaviour

er work with olfaction in fishes Wrede (1932) erved that the skin mucus from the minnow oxinus laevis Ag.) contained an attractive subnce Todd et al (1967) gave evidence that the low bullhead (*Ictalurus natalis*) recognize indiuals of their own species *from substances pres*in the skin mucu by *sense of smell. Skin* cus has also been thought to be the source of e specific odorous substances in the homeward gration of anadromous salmonids (Nordeng 1971 77). This last suggestion has been supported by ectrophysiological studies by Døving et al (1973 74) on the olfactory sense of the arctic char (Salalpinus L.) Potent olfactory substances seem to *present also in the skin mucus of other teleost* ecies (Salmo gairdneri Coregonus clupeaformis d Carassius auratus) as demonstrated by Hara & cDonald (1976)

The skin mucus substances of some fishes were died by Wessler & Werner (1957). A protein and contain 16 amino acid residues was *found to be* predominant component Enomoto et al. (1961) und only small ariations in amino acid composin of skin mucus proteins in 11 species examined. the skin mucus from Atlantic salmon Harris & ant (1973) established the presence of 18 amino id residues in dry eight sample of 62% protein ntent and an analysis of glycoproteins in skin from the char by Wold & Selset (1976) revealed 17 common amino acid residues. *No investigator has analyzed the content of free amino acids in the skin mucus which olfactory-wise would give* the most valuable information The olfactory stimulating properties of amino acids were well demonstrated by Sutterlin & Sutterlin (1971) Suzuki & Tucker (1971) Hara (1973) and Belghaug & Døving *(1977). Recent* findings by Thommesen *(1978)* of a spatial distribution of odour induced potentials in the olfactory bulb in salmonids, however indicates that a distinction must be made between amino acids and other stimulating substances

Working with skin mucus in the field of olfaction *Selset & Døving* (1980) found attraction also to intestinal contents in behavioral experiments with arctic char (Salmo *alpinus L.*) Since an extract of *food pellets* seemed nonattractant the attraction to food *might be excluded*. Our attention was then focused on the manner by which surface mucus was collected Uskova et al (1971) had shown that the utmost care has to be taken in the collection of skin *mucus Their blowing off* of the mucus with a jet of *air contra* scraping with a scalpel gave 9 amino acid *residues* in the hydrolyzed mucus proteins contrary to the usual 16. *Microscopic* examination showed that the "*customary*" method gave contamination of the mucus by epithelial cells. Together with the above findings, this gav rise to the following ques-

Table 1 *Percentage composition of low and high molecular weight material and contents of free acids in pure mucus customary collected mucus and intestinal contents from char based weight*

Material	Contents of low m wt material (%)	Contents of high m.wt material (%)	Contents of free amino acids (%)
Pure mucus	41	59	15 4
Customary collected mucus	73	?7	10 3
Intestinal contents	90	10	–

tion Could the customary method for collection of skin mucus also result in contamination by intestinal contents?

In the present study we give evidence that skin mucus collected in the customary way is indeed contaminated by intestinal contents An improved method for collecting skin mucus from fishes is introduced and the amount of free amino acids in this mucus is measured

MATERIAL AND METHODS

The skin mucus was collected from Atlantic salmon (Salmo salar L.) and arctic char (Salmo alpinus L.) All fishes were artificially hatched and raised at the Research Station for Salmonids Sunndalsora Unit Norway

A customary method for collecting mucu A fish is immobilized by a stroke on the head abo e and behind the eyes *Bleeding is avoided by using a rounded wooden* club The fish is carefully held with two fingers behind the operculum and the mucus gently scraped off from top to bottom with a knife The scraping might be repeated after *moistening* the skin surface with distilled water Squeezing out feces was avoided although small amounts of light yellow juice could sometimes be observed draining from the anal opening This method for collecting skin mucus is called the customary way in the following text I e according to Uskova et al (1971)

The developed method After immobilizing the fish it was hung from the mouth cartilage with fish hooks The skin surface was washed with a spray of distilled water The skin surface was *then vacuum-cleaned" with an* aspirator system connected to a collecting flask The mouthpiece of the vacuum-cleaner was flattened at the end and gently rounded to avoid sharp edges Skin mucu was *gently collected* from the area anterior to and above the gut opening Since the skin mucu of a swimming fish may contain substances from the surrounding water the mucus collected by the developed method is not pure in *the chemical sense of the word* In the following context however pure" skin mucus is used as a synonym *for* "vacuum-cleaned" skin mucus Intestinal content were removed from the same fishes as were acuum-cle Since fish food rests had been shown to be of no mo our work (see introductory part) the gross amo feces was eliminated by fasting the fishes for before the *material was collected by our* method The intestine was cut posterior to the p caeca and close to the gut opening A small a distilled water was introduced into the closed int *and after some minutes the mucus cont* squeezed out

Examination of the material Acetic acid was add pure mucus intestinal contents, and customary lected mucus to a final concentration of 5% This pr dure precipitates the high molecular weight mater might be used as an alternative method to isolation of fish odorants (Selset 1980) The mixtures centrifuged at 25 000×g and the UV absorption spe the supernatants were taken in the area 40–400 nm lyophilization the dry weight was measured for supernatant and precipitate of pure mucus and nal contents together with the dialysate and re customary collected mucus The *supernatants fro* pure mucus (2) intestinal contents and (3) ary collected mucus were chromatographed Sephadex G-25 column Elution with 5% acetic acid *material with absorbance at 280 nm*

2 1 ml mucus collected from the skin by the cleaning method was transferred directly into solution of ml 10% TCA The content of free acids was then analyzed on a Bio Cal BC 200 Amino Acid Analyzer using the regular protein hyd sate program for one column Mucus collected customary way was first dialyzed with a cut-off proximately 12 000 Dalton nd the dialysate wa *for its content of free amino acids. The dialysis* used w s from A H Thomas Co (Cat No 4465-110

RESULTS

The dry weight contents of low molecular weight material in the pure mucu has a lower v compared to the *high molecular weight mate* (Table 1) In the material testina

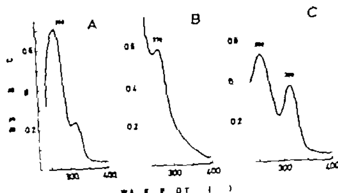


Fig. 1. UV-absorption spectra, 240–400 nm, for the low molecular weight material from Atlantic salmon. (A) Pure skin mucus, (B) intestinal contents, (C) "Customary collected skin mucus.

...w molecular weight material predominates. ...composition of low and high-molecular weight ...ial of customary collected mucus shows in...ediate values between pure mucus and intes... contents. The values obtained for the cus...ry collected mucus are not directly compara...o those for pure mucus and intestinal con...s since two different method for fractionation ...e used. However if precipitation had been used ...the customary collected mucus, the portion of ...molecular weight material would have been if ...erent, still higher than the value obtained since ...dialysate contained no acetic acid precipitable ...terial.

...he UV absorbance spectra of the low molecular ...ight material are presented in Fig. 1. The absorb... spectrum of the supernatant from custom... mucus (Fig. 1C) differs from that of pure ...cus (Fig. 1A) with respect to λ_{max} at 260 nm and ...) nm relative to λ_{max} at 268 nm and 318 nm. This ...ult might be due to contamination by epithelial ...ls as postulated by Uskova et al. (1971). The ...pernatant from intestinal contents has only one ...$_{max}$ at 272 nm (Fig. 1B).

...Gel filtration of the three types of supernatant are ...esented in Fig. ... In an ordinary gel filtration, ...e substances are eluted between the void volume ...) and the salt volume (V_s). The gel filtration of ...e material in this work produces peaks after the ...lt volume ... retarding effect known partly to be ...e to aromatic compound (Anonymous 1974). ...e gel filtration of the supernatant from pure ...mucus gives 5 peaks after the salt volume (Fig. A) ...) indicated with Roman numerals in the figure. ...e supernatant from intestinal content however

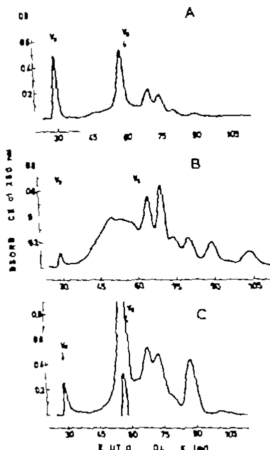


Fig. 2. Elution diagrams from the Sephadex G-25 chromatography of the low molecular weight material from Atlantic salmon found after precipitation with acetic acid. (A) Pure skin mucus, (B) intestinal contents, (C) "Customary" collected skin mucus. V_0 = void volume. V_s = salt volume. Special retarded peaks are marked with roman numerals. Note that the elution speed in the three diagrams is different.

Table 2 *Concentrations of free amino acids found in "pure" skin mucus from char*

Amino acid	Conc (nmol/ml)	Amino acid	Conc (nmol/ml)
Asp	179 8	Met	35 6
Thr* + Ser* +		Ile	40 1
Asn + Gln	49 7**	Leu	73 5
Glu	145 8	Tyr	138 0*
Pro	Trace	Phe	154.5*
Gly	75.2	Lys	51 1
Ala	103 4	Arg	31 4
½Cys	Trace	His	98.8*
Val	100 8		

Amino acids found by Uskova & Chaykovskaya (1971)
Insufficient separation for individual quantification (See examination of material)
* Possible disturbance from hexoseamines
Perhaps also methylhistidine

shows 6 peaks after the salt volume (Fig 2B) The same 6 peaks are also found in the gel filtration curve from a supernatant of "customary" collected mucus (Fig 2C) This result suggests that the "customary" collected skin mucus is contaminated with intestinal juice The dry weight values for "customary" collected mucus (Table 1) are more like those obtained for intestinal contents than for "pure" mucus Since the relative volume of intestinal contents in "customary" collected mucus can be assumed small the result can only be explained if the total dry weight per volume of intestinal contents is considerably higher than that of "pure" mucus

The amino acid anlysis revealed 19 different common free amino acids in "pure" mucus The data also suggest a considerable amount of taurin and maybe also of phosphoserine The characteristic peaks for the two substances fuse in the elution program used making discrimination difficult Threonine serine asparagine and glutamine are insufficiently separated and cannot be quantified individually In addition thryptophane may also be present but could not be measured in the system used The identified amino acids are listed in Table 2 together with their individual concentrations

On a dry weight basis the amount of free amino acids in "pure" skin mucus is higher than in "customary" collected mucus (Table 1) The content of amino acids in intestinal contents was not measured However it may be assumed very low because of both intestinal and bacterial absorption

DISCUSSION

The gel filtration of this work shows that the [...] tinal contents (Fig 2B) contain substances [...] give a retarded peak no VI not found in the [...] skin mucus (Fig 2A) collected by the [...] method Since skin mucus collected in the [...] tomary" way also shows a similar retarded p[...] this points to a contamination from intestinal [...] The UV absorbance spectra indicate a contr[...] tion also from other sources Dead and [...] epithelial cells naturally make a contribution [...] "pure" mucus Peak no VI however shows [...] absence in the gel filtration of "pure" mucus [...] all fasted groups examined This points to the [...] clusion that the origin of peak no VI in the [...] tomary" collected mucus is not the epithelial [...] The fasting of the fishes before the collection [...] material by the new method also presents an [...] tainty compared to the "customary" colle[...] mucus from normal fed donors The difference [...] handling might give a possible explanation of [...] variation in magnitude of the gel filtration [...] between the collection methods used The [...] does not however explain the total absence of [...] no VI in "pure" mucus since this peak is [...] the intestinal contents from the same fishes [...] cerning olfaction the present work therefore [...] that the utmost care has to be taken in the colle[...] of odourant material from fishes Contamination [...] the type shown are likely to be even more de[...] able by the fish olfactory organ than by most che[...] cal methods Working with skin mucus as a so[...] of olfactory material the demonstrated method [...] collection seems at present to be the best

The present amino acid analysis of the [...] mucus collected by the "vacuum-cleaned" me[...] reveals that nearly all the common amino acids [...] found as such Uskova & Chaykovskaya (19[...] found by thin layer chromatography only 10 a[...] acid residues in the hydrolyzed skin mucus [...] tein but thin layer chromatography is not as se[...] tive as a modern amino acid analyzer In addi[...] the free amino acids do not necessarily [...] from the mucus proteins since epithelial cells [...] and hemolyze at the tissue surface Anyhow [...] highest concentration of amino acids revealed [...] our experiments are mainly in accordance with [...] results of Uskova & Chaykovskaya the a[...] acids found in their experiment are marked with [...] asterisk in Table 1

sults established through the above experi- give a background for the following question: skin mucus be source of olfactory sub- s in fishes To answer this question, a brief s must be given of skin mucus sampling ods used in prior reports.

ede (193.) collected the mucus for her work by ng the fishes in a net above water for some and then sampling the mucus from the net leng (1971) and Døving et al. (1973 1974) col- d the mucus by carefully rubbing anaesthetized ly-killed fishes followed by washing with wa- H Noreng, K B Døving Personal communi- ons), Hara & MacDonald (1976) washed the surface of the fish with a water jet. All these pling methods may give contamination by intes- juice. Todd et al (1967) collected the mucus their work by wiping the dorsal surface of the with a piece of wet cloth This method for pling skin mucus seems fairly good and the k seems to establish reliable evidence for the ence of specific odourous substances in the mucus at least for the particular species used ever the authors remarked that the responses e less intensive to neutral water enriched by the or slime than to the donor water itself Lack of l residues or urine in the mucus-enriched water proposed as an explanation for the lower re nse

tema et al (1973) ha e reported that certain low ecular substances (less than 1000 Dalton) from home stream water act as olfactory stimulants lewives (Alosa pseudoharengus) The absence detectable amount of amino acids in the water cated that free amino acids were not invol ed in homestream response

he present experiment establishes the presence free amino acid in the skin mucus of fishes ce the olfactory timulating properties of amino ds are ell demon trated there can therefore be doubt that the skin mucus is source of olfactory mulating substances It is difficult however to ue that amino acids has any signal properties for food detection The pecific olfactory mulating substances which ha e been demon- ated by some of the authors mentioned may have their origin in the skin mucus We are not to see however that any definite proof has n given for the skin mucus alone acting as a urce of such substances. The present work gest further investigation in this field

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Table 2 *Concentrations of free amino acids found in pure skin mucus from char*

Amino acid	Conc (nmol/ml)	Amino acid	Conc (nmol/ml)
Asp	129 8	Met	25 6
Thr* + Ser* +		Ile	40 1
Asn + Gln	249.2*	Leu	73.5
Glu	145.8	Tyr	138 0[b]
Pro	Trace	Phe	154.5[b]
Gly	75	Lys	51 1
Ala	103 4	Arg	31 4
½Cys	Trace	His	98 8[c]
Val	100 8		

Amino acids found by Uskova & Chaykovskaya (1971)
Insufficient separation for individual quantification (See examination of material)
[b] Possible disturbance from hexoseamines
Perhaps also methylhistidine

shows 6 peaks after the salt volume (Fig 2B) The same 6 peaks are also found in the gel filtration curve from a supernatant of customary" collected mucus (Fig 2C) This result suggests that the "customary collected skin mucus is contaminated with intestinal juice The dry weight values for "customary" collected mucus (Table 1) are more like those obtained for intestinal contents than for "pure mucus Since the relative volume of intestinal contents in "customary collected mucus can be assumed small the result can only be explained if the total dry weight per volume of intestinal contents is considerably higher than that of pure" mucus

The amino acid anlysis revealed 19 different common free amino acids in "pure" mucus The data also suggest a considerable amount of taurin and maybe also of phosphoserine The characteristic peaks for the two substances fuse in the elution program used making discrimination difficult Threonine serine asparagine and glutamine are insufficiently separated and cannot be quantified individually In addition thryptophane may also be present but could not be measured in the system used The identified amino acids are listed in Table 2 together with their individual concentration

On a dry weight basis the amount of free amino acids in "pure skin mucus is higher than in customary collected mucus (Table 1) The content of amino acids in intestinal contents wa not measured However it may be assumed very low because of both intestinal and bacterial absorption

DISCUSSION

The gel filtration of this work shows that the [illegible] tinal contents (Fig 2B) contain substances [illegible] give a retarded peak no VI not found in the [illegible] skin mucus (Fig 2A) collected by the new method Since skin mucus collected in the [illegible] tomary way also shows a similar retarded p[illegible] this points to a contamination from intestinal [illegible] The UV absorbance spectra indicate a con[illegible] tion also from other sources Dead and [illegible] epithelial cells naturally make a contribution [illegible] "pure" mucus Peak no VI however shows [illegible] absence in the gel filtration of pure mucus [illegible] all fasted groups examined This points to the [illegible] clusion that the origin of peak no VI in the [illegible] tomary collected mucus is not the epithelial [illegible] The fasting of the fishes before the collection [illegible] material by the new method also presents an [illegible] tainty compared to the "customary col[illegible] mucus from normal fed donors This difference [illegible] handling might give a possible explanation of [illegible] variation in magnitude of the gel filtration p[illegible] between the collection methods used The [illegible] does not however explain the total absence of p[illegible] no VI in "pure mucus since this peak is fo[illegible] the intestinal contents from the same fishes C[illegible] cerning olfaction the present work therefore [illegible] that the utmost care has to be taken in the coll[illegible] of odourant material from fishes Contamination [illegible] the type shown are likely to be even more de[illegible] able by the fish olfactory organ than by most c[illegible] cal methods Working with skin mucus as a [illegible] of olfactory material the demonstrated method [illegible] collection seems at present to be the best

The present amino acid analysis of the mucus collected by the vacuum-cleaned" m[illegible] reveals that nearly all the common amino acids [illegible] found as such Uskova & Chaykovskaya [illegible] found by thin layer chromatography only 10 [illegible] acid residues in the hydrolyzed skin mucus p[illegible] teins but thin layer chromatography is not as se[illegible] tive a a modern amino acid analyzer In add[illegible] the free amino acid do not necessarily orig[illegible] from the mucus proteins since epithelial cells [illegible] and hemolyze at the tissue surface Anyhow [illegible] highest concentration of amino acid revealed [illegible] our experiments are mainly in accordance with [illegible] results of Uskova & Chaykovskaya the a[illegible] acids found in their experiments are marked with asterisk in Table 1

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[Che]mical methods for fractionation of odorants [pro]duced by char smolts and tentative suggestions [for] pheromone origins

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SELSET R. Chemical methods for fractionation of odorants produced by char smolts and tentative suggestions for pheromone origins. Acta Physiol Scand 1980, 108. 97–103 Received 24 April 1979 ISSN 0001-6772. Institute of Zoophysiology University of Oslo, Blindern, Norway

Chemical procedures for fractionation of fish material containing odorants are described. These methods were developed with the aim of retaining the odorous activity of the material. Some of these methods were used to fractionate the intestinal contents of 1 1 year old smolts of population of anadromous char (Salmo alpinus L.). The fractions were prepared ( ) for behavioural experiments to study possible attraction effects on mature char of the same population during the migration season, and (b) for testing in physiological experiments to elucidate the olfactory potency of the different fractions. The nature and origin of the attractive substances are discussed.

Key words Fish, salmonid smolt, intestinal contents, chemical fractionation, odorants, pheromone bile acids, micro organisms

[Anad]romous salmonid fishes migrate from their [feed]ing areas in the river systems out to sea and [retur]n to the same spawning sites as used by their [paren]ts (Clausen Fries 1599). It has been shown [that] the olfactory sense s necessary for their cor[rect] return (Wisby & Hasler 1954 Groves et al. [1968] Toft 1964) Hasler 1968 and Hasler et al 1978 [prop]osed that correct return is based upon prior [impr]inting to substances from plants and minerals [char]acteristic of the home stream water Nordeng [(1971,] 1977) has proposed a pheromone [hypo]thesis for migration of the anadromous sal[moni]ds, char (Salmo alpinus L.), trout (Salmo trutta [L.) an]d Atlantic salmon (Salmo salar L.). Accord[ing to] this hypothesis, the homeward migration is an [inna]ted response to trails of population specific [subs]tances released from the fry and descending [smol]ts of the same population

[Fo]llowing the hypothesis of Nordeng, mature [char] should be attracted to odorants produced by [smol]ts of the same population during the season for [hom]eward migration It should therefore be possi[ble t]o isolat such substances and to establish their [attra]ction effects and olfactory potency In the [presen]t study different chemical method are described which were found suitable for fractionation of the biological material from smolts for subsequent testing in behavioural and physiological experiments

MATERIAL AND METHODS

Material. The material used in the present study was collected from 1 1 year old smolts of a population of anadromous char (Salmo alpinus L.) hatched and raised at the Fish Breeding Experimental Station at Sunndalsøra in Western Norway but originating from Lake Storvatnet near the city of Hammerfest in Northern Norway (70°40′N 23°42′E) All available information evidences the existence of only one population of anadromous char in this lake (Nordeng, pers. comm.). During breeding at the Fish Breeding Experimental Station the population was kept genetically clean.

Handling of the material Since the olfactory system of the char is highly sensitive and since the postulated pheromones are expected to be present in minute quantities, great care was taken to void contamination of the material Therefore no solvent, reagent or other material that would add odorants to the biological material or disturb the olfactory system of the fish, was used unless it could be removed completely after use. T fulfil these requirements all solvents and material that were brought into contact with the odorants were tested on the olfactory

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e 1 *Final results of the behaviour tests on mature Hammerfest sea-char for attraction to material ted from smolts of the same population*

probability values are correct only if the binomial model is valid for the experiments

solution	Total no. of trials (N)	No of successful trials (X)	Probability in % for X or more successful trials of N trials
merfest sea-char smolt	313	19	<0 01
merfest sea-char smolt, stinal contents (HA)	261	165	0 01
merfest sea-char smolt, mucus	183	99	56
	18	123	3 4
	105	55	35
(	150	79	28
	210	119	4 3
3	71	23	99*
A (1 through 4)	123	66	1
-B-1	42	13	99*
B-	41	25	50
-B 3	114	68	2.4
-B-4	51	29	20
-B-5	33	16	58
B-6	31	10	96

pellent effect of the fraction

on at 40°C to remove acetic acid and was then stored 30°C The other portion as fractionated further

alternative to the precipitation method dialysis be used th tubing from A H Thomas Co (Mw off 12 000 Dalton, Cat No 4465-H02, EDP 787-022) prewashed according to the procedure of he (1971) This washing procedure removes all odor substances from the tubing Dialysis is however more timeconsuming and much more hazardous respect to microbial growth than the precipitation od

hadex G-25 chromatography The supernatant -A) as chromatographed in 10 ml portions on adex G-25 (fine) column (Pharmacia Fine Chemicals) 3 cm elution speed 110 ml/h, fraction volume 6 3 (14°C using 5% HAc in ater eluent The elution ss monitored by Bio-Rad Model 1300 UV ter set at 280 nm The collecting tubes ere pooled rding to the peak in the elution diagram and acetic as removed as described above Fractions were ed at 20°C

sorption chromatography on Amberlite XAD-2 The material of the fraction called HAS- (Fig 2) was olved in 1 ml of ater and 9 ml of 0 1 M NaOH 6 (Nakano & Sjøvall 1977). The solution was atographed on an Amberlite XAD-2 column (I D 44 1 cm, elution speed 3 ml/min) at 4°C The was prewashed with 10 olumes each of methanol, ne and ater Substances not adsorbed (HAS- -A) shed out with 150 ml of ater and taken to dry by rotatory ev poration. NaOH was not removed. material (HAS- -B) was eluted ith 150 ml of methanol, taken to dryness as above and stored at −20°C.

Sephadex LH-20 chromatography HAS-2-A and HAS- -B were dissolved each in 1 ml of methanol for chromatography on a Sephadex LH-20 column (70 1 cm. elution speed: 17 ml/h, fraction volume: 2 ml) and eluted ith methanol at 4°C. Most of the NaOH in HAS- -A was not dissolved in methanol and as centrifuged off The elution progress was monitored in the same way as abov The collecting tubes were pooled as indicated in the elution diagram (Fig. 3), and the fractions were taken to dryness and stored at −20°C

Thin layer chromatography 10 10 cm thin layer silica plates (DC-Alufolien Kieselgel 60 F 254 0.2 mm, Merck Art 5554) were used with the solvent system chloroform/methanol/25% NH in the volume ratio 2/2/1 The solvent front was allowed to reach the edge of the plates. Substances were visualized by means of an ultraviolet lamp (254 and 366 nm) normally as dark spots on a fluorescing background using 254 nm UV-light. However some of the substances were themselves fluorescing when UV light of 366 nm was used.

Dry eight measurements. Aliquots of the different fractions and the starting material were taken out for lyophilization and weighing to determine the dry weight of the material

Gas-liquid chromatography–mass spectrometry To identify possible bile acids in the fraction HAS- -B-3 0 2 mg of this material as subjected to permethylation and subsequent analysis by gas-liquid chromatography–mass spectrometry as described by Almé et al (1977). The analyses ere carried out at Karolinska Institutet Stockholm by J Sjøvall in January 1979

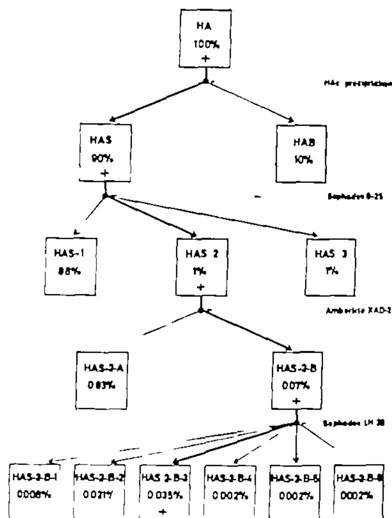


Fig 1 The mainl nes of the isolation procedure Proportions of dry weight material are given n per cent Attractive fraction are marked +

ystem of char with electro-physiological method as de scribed by Thommesen (1978) All glas equipment were wa hed with chromic ulphuric acid water 0 5 N hydrochloric acid water and then dried at 140°C All equipment to be used for the odorant wa handled with Triflex glo es (Code FKD 7073 Tru enol Laboratories Ltd) likewise tested by electro-phy iological method All w ter used was double di tilled w th a Quickfit 3DWS Double Distillation Water St ll (Corning Ltd) It w found essen tial that the double distilled w te be tested with egard to olfactory potency at regular nterval in case of any microbial growth This i a problem espec ally when poly ethylene torage containers are used

C llection f m terial The kin mucu f molt has been thought to be the source of the postulated migration pheromones in salmonids a reported by Nordeng (1971) and Døving et al (1973) Howeve beha ou e peri ments (Selset & Døving 1980) on anadromous cha with pure skin mucus collected with new method (Stabell & Selset 1980), and intestinal content re aled that only the l tter was a source of substances attract ng matu e cha during the migration se son The chemi al study was therefore f cused on the intestinal cont t of cha

molt The large intestine from the phylorıc cae gut opening of 30 newly killed fishes were cut ... content (0 1 1 0 ml per fish) squeezed directly ... ml entrifuge tubes The volume of each tube justed to 8 ml by addition of water The tubes were vigorously and particles were centrifuged off at ... in Sorvall Superspeed RC -B automatically refr centrifuge for 30 min at 0°C The supernatan pooled and used uring material for the exper

P ecipitat n of h gh molec lar eight material ... cetic acid was added to the pooled supernatant (HA) to a final c ncentration of 5 ... By compar f und that all the high molecular weight material that w retained n dialy bags with a molecular cut-off of pprox mately 1 000 Dalton and ... some other substance a well precipitated ... method The prec pitate w centrifuged off as de abc e nd wa hed with about 50 olumes of 5 acid 3 time nd once with w ter The precipitate was the lyophilized nd tored t 20°C until ... supernatant HAS was d ided into two portion portion wa taken t dryness 3 t mes wit ... water in a rotatory ev chi l

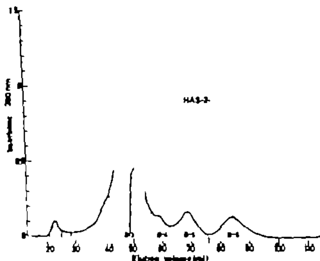


Fig 3 Elution diagram for Sephadex LH 20 chromatography of the fraction HAS-2-B The upper part of the peak HAS- -B-3 is inserted in its base (max. abs 3 0).

char seemed to behave indifferent towards the r fractions

e chromatographic procedures used in this t seem to give only small loss of material. The al los could only be estimated because some of fractions were difficult to dry

he fraction HAS- -B 3 was found in the siological tests, to have a threshold value for ctory response that was a 100 times lower than other fraction. In fact the odorants in this tion seemed to be as potent as bile acid deriva- s which are the most powerful odorants found fishes so far (Doving, Selset & Thommesen 0)

he results of some of the thin layer chromato- ms of the different fractions are seen in Fig. 4 In figure are shown the spot patterns found by ication of HAS-2, the XAD-2 fractions A and B the ubfractions of HAS-..-B from the LH-20 umn The HAS- -B-3 chromatogram shows six ts of which three are fluorescent at 366 nm. Five these spots may be due to substances also pres- in neighbouring fractions. The nature of the ts in this chromatogram suggests that HAS-2 contains mainly 3 different substances, the non- orescing spots The gas-liquid chromatography— spectrometry analyses showed with a high gree of reliability that cholic acid is the main mponent of HAS-2-B-3 Cholic acid has an Rf TLC system used that coincides with

the second nonfluorescing spot of HAS-2-B-3 A second main component was believed to be some kind of branched hydrocarbon which was not fur ther identified A third substance was tentatively identified as dioctylphtalate (plasticizer) a normal contaminant of such systems. These three sub- stances are believed to be the cause of the three nonfluorescent spots in the chromatogram of the fraction HAS-2-B-3 No further investigations into the structure of the fluorescent minor components of this fraction have been carried out so far

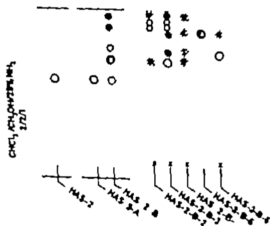


Fig 4 Thinlayer chromatograms of some of the fractions obtained Dark spots (see text) are marked with open circles. Fluorescent spots are hatched

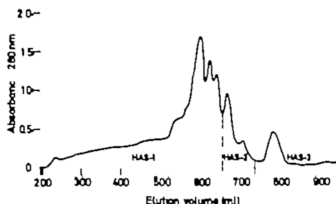


Fig 2 Elution diagram for Sephadex G-25 chromatography of the supernatant HAS The salt volume was 565 ml

RESULTS

The outlines for chemical fractionation used in the present study were governed by results from the behavioural studies These studies were made during the period of homeward migration normal to mature char of the wild population These results are described in a subsequent article (Selset & Døving 1980) Additional information for the chemical work was obtained by studying the physiological effects of the chemical fractions on the olfactory organ of the char

The diagram in Fig. 1 gives the main lines of the isolation procedure used in the present study High molecular components of the intestinal contents were precipitated The attractive supernatant was first fractionated on Sephadex G 25 and further by the use of Amberlite XAD-2 Finally the material was fractionated with a Sephadex LH 20 column The fraction HAS 2-B 3 most likely contained the attractive substances This fraction contained 0 035% of the water soluble part of the intestinal contents The initial fractionation of the intestinal contents (HA) by precipitation probably results in a precipitate contaminated with low molecular material because of adsorption effects but this does not include the substances attracting mature char as determined by behavioural experiments The attractants seem to be present only in the supernatant (HAS)

The final results of the behavioural experiments (Selset & Døving 1980) with their respective probability values calculated using the binomial model are presented in Table 1 These data are presented more comprehensively in the referred article

The supernatant was fractionated further on a Sephadex G 25 column The elution diagram (Fig 2) shows an elution process that deviates markedly from ordinary gel filtration in that some of the material is strongly retarded Especially aromatic substances are known to be retarded in this way (Janson 1967)

Originally the eluate from the G-25 column was divided into 7 fractions according to the peaks in the diagram but as the behavioural experiments established the presence of attractants only in the fraction called HAS-2 in Fig 1 the other fractions were pooled as shown in the figure The bulk of amino acids were eluted just before the salt volume (V_{NaCl}=565 ml) The attractive fraction HAS-2 contained only 1% of the starting material (HA) by weight HAS-2 is one of the retarded fractions In a concentration of 1 g/l in water it also forms micelles that are clearly visible on cooling the solution This means that HAS-2 could contain lipids that also might possibly be of aromatic character This fraction has a relatively high absorption of ultraviolet light in the range of 280 nm The electro-physiological responses recorded from the olfactory bulb induced by HAS-2 had similar properties to those obtained by stimulation with bile acids and derivatives (Døving, Selset & Thommesen 1980) These facts indicated that the attractants could be some kind of bile acid derivative For this reason the next fractionation step chosen to be adsorption chromatography on a column of Amberlite XAD-2 a resin known to adsorb amongst other compounds bile acids More than 90% of the HAS-2 contents (called HAS-2 A) passed right through the column while the rest (HAS 2-B) was adsorbed to the resin Whether or not methanol will release completely all the substances adsorbed is not known but the attractants seem to be eluted by this solvent

Further fractionation of HAS-2 A and HAS-2 B was accomplished by use of the lipophilic Sephadex LH 20 with methanol as eluent Fairly good fractionation was achieved of the HAS-2-B contents (Fig 3) whereas the HAS 2 A substances were not separated well by this method The fraction HAS ... contained the rest of the NaOH originally ... and no other substances as judged by thin-layer chromatography and pH tests and therefore NaOH presented no problem to the electro-physiological and behavioural experiments According to the behavioural experiments the fraction HAS 2 B 3 should contain the attractants

r and secreted into the bile but it seems
...dous then to transport them the long way
...ugh the intestine where they will be exposed to
...bial attacks even though the intestinal mi-
...ganisms seem to be much less numerous in
... than they are in mammals (Sacquet et al
...) The exact identification of the structures and
... of these substances require further in es-
...ion

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DISCUSSION

The present study gives examples of chemical methods we believe effective in handling odorant containing material from fishes in such a way that the biological activities of the odorants are likely to be retained The methods used are also suitable for physiological tests on the olfactory organ The main topics discussed are the origin of the attractants the precautions necessary for their biological effects to be retained the chemical nature of the attractive substances and the consequences of the chemical nature of such substances in the life history of migratory salmonid fishes

The material studied in the present investigation was intestinal contents of char smolts sampled at the beginning of June This period was selected since it coincides with the season for homeward migration of mature char (Nordeng 1977) The smolts were from the in-bred population of chars raised at the Fish Breeding Experimental Station and not from the wild population It is not known if the production of attractants are restricted to the migration season It is also uncertain if the postulated phermones will be produced at all in an artificially raised population However we have shown that the smolts do produce substances that attract the mature chars during the migration season (Selset & Doving 1980)

In chemical work with such odorants it is of the utmost importance to avoid any sort of odour contaminations since there is no way of knowing in advance if they will affect the behaviour of the fish It should be mentioned here that extracts of human skin have been reported by Idler et al (1956) to induce fright reactions in some salmonid fishes Great care should be taken to prevent microbial growth Bacteria will be present in the starting material (Yoshimizu et al 1976) and may rapidly transform many of the interesting substances (Hill 1976) into derivatives not produced in the intestine under natural conditions Certain bacteria encountered in this work have been shown to produce odorants inducing electro-physiological responses similar to those of fraction HAS-? B 3 and bile acids and with similar threshold values

Precipitation of the high molecular weight components of HA did not result in a fractionation of the material into odorants and nonodorants presumably because of adsorption effects as already mentioned Bile acids and derivatives mostly microbially transformed are known to be present in considerable amounts in the large intestine ... potency as odorants (Doving Selset & Th... 1980) and their detergent effects could ex... precipitate being a definite although weak... stimulant

The chemical structure of the attractive substances can partly be deduced from the ... information The attractive fraction was re... on the G 25 column indicating aromatic co... nents At high concentrations the fraction f... micelles which indicates presence of polar ... The attractive substances were adsorbed ... XAD-? resin These facts make it likely th... attractants are some sort of polar steroid mole... possibly containing aromatic rings

The main component of HAS- -B 3 was ... by Sjowall to have the same retention time a... and the same mass spectrum as cholic acid ... indeed a polar steroid Cholic acid is produ... all salmonids (and many other fishes as well ... ti & Sidorov 1973) and can therefore not func... a population specific pheromone However ... acid is shown to be a strong odorant for ... (Doving Selset & Thommesen 1980) and possibly be part of a multipheromone sys... homing in salmonids

The other main components of HAS- -B ... dioctylphtalate and the branched hydrocarb... not likely to be either attractants or odoran... fishes

The minor components of the fraction ... fluorescent substances have not yet been ... tified but these could turn out to be polar ar... steroids

The bile acids known to have impor... physiological roles as detergents in the diges... fat and to facilitate the uptake of certain metab... most likely be adsorbed to organic matter and ... erals at the spawning sites in the water stream ... rate of adsorption and release will then depe... physical factors like temperature water flow ... etc and the stream s composition of organic ... and minerals

There is a theoretical possibility that these ... stances can be retained at the spawning site l... enough to secure homing for the fish popu... even if the fishes are absent for a period of t... (e g Pink Salmon) (Harden Jones 1968)

The origin of the attractants is uncertain T... *may be produced by microorgani m in the* ... tine from bile acids They ... also be produce...

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don of α-MSH on the release of neurotransmitters m the retina

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ence is now rapidly accumulating that there are y classes of neurons in the CNS that operate peptides as neurotransmitters or neuromodu- s and there is a reasonable chance that α-MSH be such a peptide mainly because it seems e present in certain specific neurons (Désy elletier 1978, Dubé et al 1978 Pelletier & é 1978 Jacobowitz & O'Donohue 1978) There of yet no clearly defined function for α-MSH in rain, but over the years, many behavioral and iological effects of it have been described (Kas- t al 1976, Tilders et al 1977) including the eye naoka 1951) In the course of a study of possible al neurotransmitters we have therefore tested effect of α MSH on the release of retinal neuro- mitters and found profound effects which are orted here

laterials and Methods Eyes of pigmented rab- weighing 1–2 kg were used. 10 or 20 μCi ated glycine, GABA, 5-hydroxytryptamine or amine were injected intravitreally and the ani- was killed by an i. air injection 1 or 4 h later posterior half of the eye was everted and unted on a glass dome in a small thermostated °C) chamber (Bauer 1977) and drip superfused (1 min) with a balanced salt solution according to rland & Ames (1976) equilibrated with 5% CO_2 95% O_2 Synthetic α-MSH (CIBA batch 61-Ba) was applied dissolved in the same solu- and always equilibrated with the gas mixture at least 20 min at 37°C before application. radioactivity of the superfusate was monitored 1 min samples. It has previously been shown t under these circumstances most of the radio- vity of the retina represents the unchanged nsmitter [³H]-glycine [³H]-GABA or [³H]- mine in the respective cases (see Kramer 1 Bauer & Ehinger 1978). There is also evi- nce that most of the [³H]-5-hydroxytryptamine ken up into the retina remains as such (Ehinger published). Therefore any increase in the release of radioactivity most likely reflects a release of the unchanged transmitter even if it may appear in the effluent as a metabolite In particular this is the case for GABA (Bauer 1978). This assertion was corroborated in a few studies on the release of radioactivity from rabbits injected intravitreally with [³H]-dopamine or [³H]-5-hydroxytryptamine and treated with the monoamine oxidase inhibitor Pargyline (50 mg/kg i.p 4½ h before the start of the superfusion). The effects of α-MSH were in these cases indistinguishable from that seen in animals not pretreated with Pargyline

Results and comments α-MSH induced a strik ing increase in the release of radioactivity from retinas which 4 h previously had been given either [³H]-dopamine or [³H]-GABA The concentrations needed for a small but still discernible response in a single expt. was about 10⁻ M. The effect increased with increasing doses. At 0.6×10^{-6} M α-MSH had pronounced effects on retinas loaded with [³H]-GABA or [³H]-DA (Fig. 1*a* and *b*) but no signifi- cant effect on retinas given [³H]-glycine or [³H]-5- hydroxytryptamine

It was previously shown that at short times (½–1 h) after the intraocular injection of [³H]-GABA, the localization of radioactivity is mainly in the glial cells (Ehinger 1977) whereas the localization is in neurons at 4 h. This can be used to test whether the source of increased efflux or radioactivity is mainly from nerve cells or glial cells. α-MSH (0.6×10^{-6} M) was found to have no effect when applied to retinas taken only 1 h after the injection of [³H]-GABA, and it is thus most likely the neurons which are the source for the increased efflux of radioactivity in- duced by α-MSH

It is noteworthy that the concentration of α-MSH needed to get effects is as low as around 10⁻ M or less. The excitant amino acids, glutamic acid and aspartic acid, which rank as the strongest exciters, have discernible effects in our test system down to 10^{-6} M (Bauer 1977) α-MSH in our hands thus

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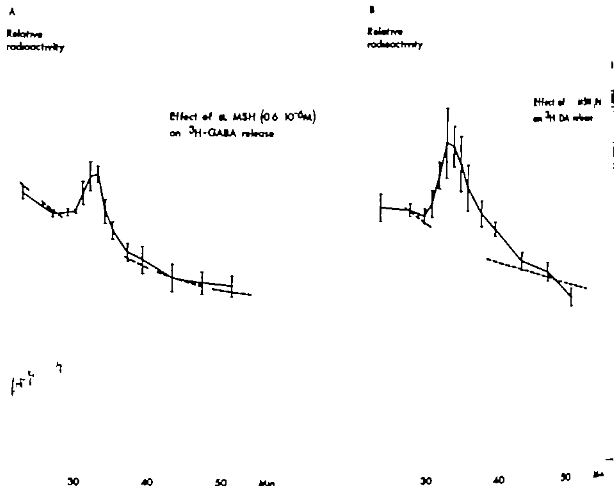


Fig 1 a Effect in vitro of α MSH 0 6×10⁻⁶ M on the release of radioactivity from 4 rabbit retinas loaded in vivo μCi [H]-GABA intravitreally Superfusion was started 4 h after the injection and the time given on the X axis is after the start of the superfusion α MSH was applied for min as indicated The individual release curves from the were normalized as described previously (Bauer & Ehinger 1978) and the vertical bars indicate the standard error of mean for the individual points The normalization procedure transforms the radioactivity measurements into c but relative units and there are therefore no scale markings on the y axis The unstimulated control efflux is repr by the dashed curve The change in release rate induced by α MSH is significant ($p<0\ 001$ calculated as the dif of slopes of the curve segments between 30 and 33 min)

Fig 1 b Experiment as in Fig. 1*a* but with [H]-dopamine labelling of retinal neurons The change in relea induced by α-MSH is significant ($p<0\ 001$ calculated as the difference of lopes of the curve segments between 3 33 min)

ranks as the hitherto most powerful retinal exciter which raises the suspicion it is a neurotransmitter or a neuromodulator in the retina

The site of action of α MSH is not known but the experiments permit certain conjectures As noted above it is likely to be on neurons rather than glia Further if its action were at the level of the photoreceptor synapses it would seem likely to be detectable in all or most cells in the inner plexiform layer i e it would be expected to be about the same on dopaminergic GABA-ergic glycinergic or indoleamine accumulating neurons which all are located in the inner nuclear and inner plexiform layers in the rabbit Since the effect of α-MSH is not equal on these neuron types it seems that α-MSH a the second synapse in the inner plexiform l Here dopaminergic neurons are contacted m by amacrines (Dowling & Ehinger 1978) wh the indoleamine accumulating ones are conta almost exclusively by the bipolar cells (Ehing Holmgren 1979) We therefore guess that if α-M is a neurotransmitter or neuromodulator i retina it is released by amacrine cells rather t by bipolar cells However before these co tures can be validated or refuted more rese is needed on the concentration and localizati α MSH in the retina and on its effects o dividual retinal neurons

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iced epileptiform activity evoked from dendrites ippocampal neurones

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arization shift (DS) and spontaneous firing are nown features of the epileptic neurone DS wever not evoked when the epileptogenic ance is applied close to the soma of neocor neurones (Walsh 1971). This could be due to sitivity of the soma membrane to the epilepto- substance or to competitive effects evoked. es on single neurones have shown that epi- genic mechanisms are referrable both to d synaptic and non-synaptic membrane erties. It is, however not clear how dendritic ions are influenced in epileptogenesis. The ocampal CA 1 pyramidal cells, with separate ptic populations on dendrites are useful when igating the epileptogenetic role of dendrites erequisite is, that the applied epileptogenic tance is restricted to the dendrites. A method microinjection has recently been described by sson et al. (1979). In this report that method ed to study the epileptogenic effects of penicil- pplied to apical dendrites, resulting in action ntials recorded from the somata of pyramidal

ea-pig hippocampal slices were cut and main- d in vitro as described by Skrede & Westgaard). Pressure microejection of 0.3–1 nl 34 mmol Cpenicillin, pH 7.3 was made from glass ttes with 2 or 5 μm tips. The 34 mmol penicillin chosen to achieve a rapid effect The ejections e made distally into the apical dendrites at hs comparable to those of cell bodies recorded). Repetitive identical pressure pulses were n, during 3–10 s time, depending on the amount cted. A study on (^{14}C)-penicillin diffusion has wn that the functional changes to be reported, referrable to penicillin effects on apical den- es

ynaptic activation of apical and basal dendrites, performed with 100 μs current pulses de- livered separately via platinum wire electrodes, placed among afferent parallel fibres impinging on corresponding dendrites. The afferent input was in many slices restricted to 80 μm wide bridges (Andersen et al 1979*b*). The stimulus strength was set at the threshold for soma spike potential An elec trode for antidromic stimulation was placed upon the alveus Intrasomatic recordings were obtained with glass electrodes placed under microscopic control in the soma layer Current pulses were passed from the recording electrode. Conventional DC-re cording of responses was made The responses were photographed and analysed from film.

Records have been obtained from twelve CA 1 cells. The resting membrane potentials ranged from −50 to −65 mV and the action potentials evoked were 70–95 mV in amplitude The effects of apical dendritic microinjections were followed 5–40 min Penicillin, ~0.5 nl corresponding to 80–100 μm spherules injected distally among apical dendrites 300–400 μm from the cellbody rapidly causes de polarization, and firing of additional spikes The depolarization appears as an afterdepolarization or as a DS during injection, or a few seconds later and remains for several minutes. Typically the changes relate to the synaptically evoked response from the injected apical dendrites After injection the resting membrane potential remains virtually unaltered. The depolarization evoked in the cellbody by synaptically activated penicillin treated apical dendrites is enhanced and prolonged as compared to the generally unchanged basal dendritic response The amplitude increase is of the order of 5–10 mV and the duration of the depolarization is prolonged by 10–50 ms. The first spike potential evoked by synaptic stimulation of penicillin influenced apical dendrites is identical with that of the control but elicited at slightly shorter latency and more regular ly Additional action potentials may ride on the

nisms evoke epileptiform activity (Traub Our observations are seen also when cells are d with weaker concentrations of (^{14}C)-llin, demonstrated to be confined to the apical tes They are also in accord with those ob- from CA 1 cells bathed in penicillin (Ander al 1979 Schwartzkroin & Prince 1978)

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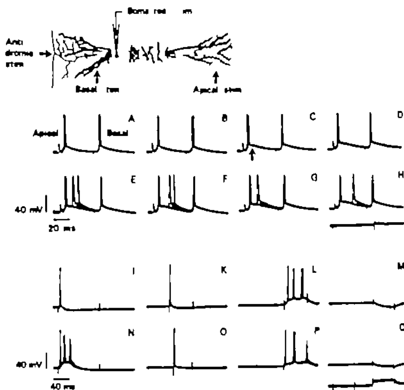


Fig 1 Inset Diagram of experimental set up showing apical and basal dendritic and antidromic activation while recording from cellbody electrically also stimulated Penicillin injection into apical dendrites shaded Intracellular records from two CA 1 cells injected with penicillin A H and I–Q with 50 and 62 mV membrane pot A control synaptic activation via afferent bridges 1st action potential evoked from apical dendrites 115 μA pulse action potential from basal dendrites 5 μA B 0 7 nl penicillin injected apically record taken 2 s later C recordings 10 and 23 s after injection show DS and extra action potentials recorded 30 s E 41 s F 90 s G, and H after injection Extracellular response shown below H I–M controls from the second cell I apical synaptic resp to a 5 μA pulse in a slice without bridges K Antidromic response to a 30 μA pulse L and M soma responses to –0 nA pulses passed through the recording electrode N–Q corresponding records taken about 3 min after injection Extracellular response shown below Q

penicillin induced depolarization Some are identical to the first one others lower in amplitude Spontaneous firing occurs at times Samples of responses obtained are shown in the Fig. 1 The tracings A–H are from one I–Q are from another cell The first neurone was synaptically activated via 80 μm wide afferent bridges placed near the apical (the first action potential) and basal dendritic segments (the late action potential) A is the control B is taken 7 s after 0 7 nl penicillin has been injected Note the change of the afterpotential 10 s after start of injection (arrow in Fig. 1 C) Records D–H show further depolarization enhancement and additional action potentials evoked only from the now "epileptic apical dendrites The responses elicited from non-injected basal dendrites may at times show minor increase in depolarization possibly due to the paired test situation Antidromic invasion is usually not influenced However as shown in recordings K and O from the second cell two antidromic responses obtained before and after t tion may show slight changes in after-hyperpolarization The synaptic response evoked via dendrites shows the typical depolarizing effect penicillin (cf I–N) Depolarizing pulses passed the recording electrode over the soma membrane showed in some cells a shortening of the latency the first action potential (cf L and P) in other the opposite effect was seen Increase in number soma spikes was not seen Hyperpolarizing injected into somata of penicillin influenced showed no or minor alterations in electrical membrane properties (cf M and Q)

The observations reported here illustrate that application of penicillin only to the dendrites of neurone is sufficient to render the cell "epileptic synaptic input The present findings fulfills the prediction from a neuronal m t altered dend

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haviour of mature anadromous char (*Salmo alpinus* L.) wards odorants produced by smolts of their m population

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SELSET R. & DØVING K. B Behaviour of mature anadromous char (*Salmo alpinus* L.) towards odorants produced by smolts of their own population. Acta Physiol Scand 1980 108. 113–122. Received 24 April 1979 ISSN 0001–6772. Institut of Zoophysiology University of Oslo, Blindern, Norway

Mature anadromous char (Salmo alpinus L.) of specific population were tested in behaviour experiments with respect to attraction effects of smolts of the same population, smolt material, and chemical fractions of that material. The char reaction indicated preference for intestinal contents and possibly bile from own population over skin mucus material and food Chemical fractions isolated from the intestinal contents were tested for attraction at concentration of 1.5 10^{-9} g per l. One of the fractions attracted the mature char The results are discussed in relation to the migration pheromone hypothesis proposed by Nordeng (1971) as being crucial for the return of anadromous salmonids to their home river' spawning grounds.

Key words Fish salmonid char behaviour smolt attractants, intestinal contents, bile, chemical fractionation, migration, pheromone

homeward migration of salmonids depend ily upon an intact olfactory organ as shown, by Wisby & Hasler (1954) Groves et al (1968) l (1975). These findings imply that the fishes are ded by odour trails back to their spawning unds. The substances responsible for these trail e been suggested to emanate from the plants and erals characteristic of the home stream water sler 1966, Hasler et al 1978). Nordeng (1971 7) has proposed that the substances are released m the smolts of the same populations as the grating fishes. Physiological experiments have wn that there are substances potent to the olfac y organ emanating from char (Døving et al. 1973 ommesen 1978) Such substances can also in- e differential responses in the cell of the olfac y bulb of chars (Døving et al. 1974)

n behavioural experiments Høglund & Åstrand 73) hav shown that juvenile chars preferred wa- r containing scent of individuals of the same the present experiment we show that romous chars are attracted to water scented by smolts of their own population and that a source for these odorants is the intestinal contents. Evidence is provided that the chars are attracted to a chemically isolated fraction of this material (Selset 1980)

MATERIAL AND METHODS

Experimental fish The fishes used for behavioural experiments were from a genetically clean population of anadromous char (Salmo alpinus L.) originating from Lake Storvatnet near the city of Hammerfest in Northern Norway (70°40'N 23°42' E). This population starts its return to freshwater at the end of June and the migration is completed at the beginning of September (Nordeng 1977 and pers. comm.). The time for return to the river depends on the age of the fish, the veterans returning first and the smolts of the year last. The fishes were hatched and raised at the Fish Breeding Experimental Station at Sunndalsøra in Western Norway (62°40' N 8°34 E) where the behavioural experiments were also performed. The 1973 and 1974 generations were offspring of the wild fish population, while the 1975 1976 and 1977 generations were all second generation in captivity

Under the conditions at the Fish Breeding Experimental

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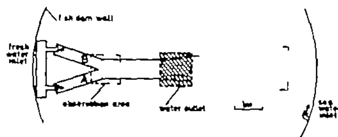


Fig 1 Sketch showing the test pen for behaviour studies on char used in 1976

Station the char reaches smoltification approximately 1½ year after hatching. After smoltification the chars were allowed to follow their natural annual sea-water/fresh-water cycle with short intermediate periods in brackish water

Until smoltification the population was kept isolated from other populations After smoltification the char no longer have the opportunity to sense the smell of fry and smolts of any population

In addition to artificially raised char smolts from the Hammerfest population anadromous char smolts from Salangen (68°51 N 18°0 E) and stationary char from Tinnsjoen Rjukan (59°45 N 9°0 E) were used as odour donor material The experiments were performed in July August and at the beginning of September between 17 00 and 01 00 hours. The natural migration takes place between 18 00 and 24 00 hours (Nordeng pers. comm)

Experimental pen In the behavioural experiments the fishes were given an opportunity to choose between two water currents one or both of which contained a solution of substances under testing. Several systems were tried two of which are described here Both systems were situated outdoors in circular concrete fish dams 10 m in diameter painted with dark green Interracing (International Fact). The water outlets were grids in the center at the bottom of the fish dam

Equal flow rates of the fresh water current to each pen were achieved by letting water flow into a wooden reservoir of about 220 liters placed just above the upper ends of the canals The bottom of the reservoir had a round hole (5 cm) for each canal In order to keep the flow rate constant at 240 l/min in each section the water level in the reservoir was adjusted by means of an overflow system and excess water outlet. The system used in 1976 was a Y maze made of untreated spruce (Fig. 1) In this system the whole dam was filled with water to a certain level

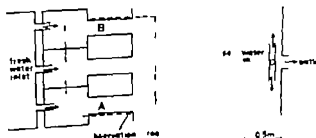


Fig 2 Sketch showing test pen used in 1977 and 1978

Table 1 *Groups of substances tested in behaviour experiments with their concentr[illegible] canal water*

The codes are explained in the text

Test solution	Concentration of dry weight material from the test solutions in canal water (g/l)
Intestinal contents	ca. 10
Skin mucus	ca 10⁻
Bile	ca. 10^{-4}
HAS-1	1.5×10^{-4}
HAS-2	1.7×10^{-4}
HAS-3	$2.3\times10^{-}$
HAS-2-A 1	$1.5\times10^{-}$
HAS-2 A	1.5×10^{-4}
HAS-2 A 3	1.5×10^{-4}
HAS 2 A-4	1.5×10^{-4}
(Combined tests)	
HAS-2-B-1	1.5×10^{-4}
HAS 2-B 2	1.5×10^{-4}
HAS-2-B 3	$1.5\times10^{-}$
HAS-2-B-4	1.5×10^{-4}
HAS-2-B 5	1.5×10^{-4}
HAS-2-B-6	1.5×10^{-4}

while with the second system only the test pen contained water The second pen (Fig. 2) used in 1977 and 1978 made of laminated wood but with canals of grey PVC wooden surface was painted with green Interracing (International Fact) and the bottom of the canals were pa[illegible] white to facilitate detection of the fishes. The se[illegible] system had three fresh water canals. The one in middle was used to secure good separation of the [illegible] trails from the other two canals. This was confirmed colour tests The Y-maze did not give as good a se[illegible] tion Most of the test compartment in the second pen was covered with dark plastic to provide protection [illegible] disturbance in the choice situation. At the upper end of canals small wooden boxes (pools) were placed to make easier for the fishes to turn The water level was kept 15–20 cm in the test compartment of both systems means of overflow arrangements

Water source The freshwater used in the reser[illegible] and canals came from a nearby river (Lillelv[illegible]) *through PVC pipelines The mature char were kept* water from this source during the winter The water [illegible] lower downstream part of the pens was kept brackish means of a sea water inlet (Figs. 1 and) with a flow of about 250 l/min in attempts to simulate natural c[illegible] tions

Test solution The solutions to be tested were ad[illegible] via polyethylene tubing to the water-streams from 5 l polyethylene containers positioned above the reser[illegible] By means of magnetic valves the flow of test solu[illegible] was controlled from a room situated outside the dam test solution were added to the water currents at a rate about 35 ml/min The turbulence brought about thorough mixing which was visualiz[illegible] colou[illegible]

Fig. 3. The variation in activity of char in the test pen during the period from 17.00 to 01.00 hours in June, July and August in 1976. Each entry gives the mean number of trials per test period of 15 min. The number of test periods are indicated to the right of the entries.

[illegible]chemical fractions tested and their concentrations in [illegible]smolts are listed in Table 1. Besides the chemical [illegible] intestinal contents, skin mucus and bile, we [illegible]d possible attraction to live fish of different strains as [illegible]. Tests were also performed with [illegible] solution of food [illegible]s used in smolt feeding. The solution was prepared [illegible] 50 ml of dry pellets partly dissolved in 5 liters of [illegible]r and filtered. This solution is approximately 10 000 [illegible] diluted in the water currents.

[illegible]ests [illegible]ere performed with living smolts as odour [illegible]ers. This was done by keeping from 3 to 6 smolts in [illegible] tank placed just outside the test pen, and an empty [illegible] at the other side for visual symmetry and leading [illegible] overflow water from the tank into one of the canals by [illegible]ans of polyethylene tubing. The concentration of sub-[illegible]ces given off by the smolts is unknown. The concentra-[illegible]tions of the solutions containing skin mucus and intesti-[illegible] contents were estimated indirectly and are uncertain. [illegible] concentration of the bile solution was estimated from [illegible]rature values and is likewise uncertain.

[illegible]ecording *of activity.* The fishes were observed by [illegible] of an infrared television system (Grundig Elec-[illegible]tronic FP 50) covering the observation area marked in [illegible] 1 and 2. Whenever complete darkness prevailed, two [illegible]frared lamps, each of 300 W mounted symmetrically [illegible] 1.5 m above the water surface were turned on. Since [illegible] test system [illegible] situated outdoors, the illumination [illegible]lowed the diurnal rhythm. It should be noted that the [illegible] light conditions at Hammerfest are quite different [illegible] those at Sunndalsøra.

[illegible]The fishes [illegible]ere considered to have made [illegible] choice [illegible], by preference, when the snout of the fish reached [illegible] marked by tape, stretched over the top of the ca-[illegible] (Figs. 1 and 2). Automatic registration with infrared [illegible] of the fishes entering the canals [illegible] attempted, but the results obtained by using these devices were found to be unreliable in the present experiments.

Experimental procedure. The solution to be tested was introduced first into canal A (Figs. 1 and 2) for 15 min., usually there was no solution added to canal B. The flow of solution was then stopped for 5 min. In the next 15 min the solution was added to canal B followed by a new pause of 5 min. This 40 min sequence was repeated until a sufficient amount of data had been obtained or no more of the test solution was available. The whole procedure constituted a test session for that particular solution. The number of fishes kept at the same time in the pen varied from 19 to 34 for the first system (1976) and from 5–10 for the second system (1977–1978). Experiments with only one fish in the pen were tried, but found inappropriate because of low efficiency. The fishes could move freely up and down the canals, one or more at [illegible] time.

Data treatment. In the experiments it was considered a trial whenever a fish entered one of the canals as described above. Since only two canals were considered for entrance and since the probability for the fish to choose one of the two canals was expected to be 0.5 and constant the binomial model was chosen for the probability calculation (see results). The results are presented as the "response value". The term expresses the number of fishes which have chosen the canal containing the test substances, i.e. "successful" trials divided by the total number of trials. The response value can theoretically vary between 1 and 0, which will signify strong attraction and repulsion, respectively. The final response value obtained in a test session considering all trials would determine the probability of attraction. These values are presented in Table 2. An extract of the table is presented in the preceding article (Selset 1980) to facilitate the understanding of the route of chemical fractionation. We

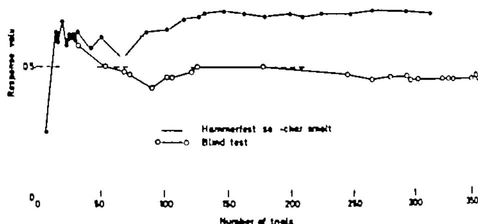


Fig 4 The response value for mature char towards the odour of their own smolts (●) and towards neutral water "added" as a test solution (O) In this and the following figures the horizontal stippled line in the diagrams represents a response value of 0.5 indicating no preference The dotted line represents approximately the response values with the probability of 1% Some of the entries represent more than one test period due to overlap in the drawings

consider such tabulated results to hide information about the development of the behaviour tests that should not be concealed from the reader In the result section graphic representation of the response values are presented in diagrams showing the cumulative results after every 15-min test period In this way results showing attraction will not be masked by later results from periods when the fishes may not be interested Due to the drawing technique overlapping entries are represented by one symbol In the diagrams the curve representing a probability limit of 1% is indicated Normally a test solution was considered attractive if the response value curve exceeded the 1% level

Environmental factors In order to enable prediction of the active periods of the fishes, several environmental parameters possibly connected with their activity were routinely noted at the start of every test These parameters were water and air temperature such weather conditions as rain cloudiness wind and light intensity Influence on activity by tide and gravity force changes induced by the tide water acceleration force was possible but could not be satisfactorily examined because the tests were performed almost exclusively between 17·00 and 01·00 hours.

RESULTS

The fishes were accustomed to the artificial breeding environment and seemed to readily adjust to the test pens During the experimental period the fishes showed wide variation in activity and willingness to enter the canals and thus to produce a trial Activity in this respect is presented in Fig 1 the mean number of trials per test period of the and shows a maximum at about 19·00 hours The mean number of trials varied greatly from day to day

Responses to live fish The response of the char to the odour of live smolts was tested. An example of the results from the test session made in 19 shown in Fig 4 In this session Hammerfest char of three years of age was tested as regards smolts of own population The response seemed indifferent at first trials but after the initial periods the results show a clear attraction The results of similar tests in 1978 were not as convincing as those from 1977 but still statistically significant at 1% level Indifferent response to the scent was never observed

Controls Several experiments were performed to observe the behaviour of the fishes with no added test solution In Fig 4 is shown the reaction in the two test canals when neutral water was added as a test solution In this blank session periods of 15 min were performed During the session the fishes made 357 trials of which 162 were made to the canal "containing" the blank Of 357 fishes (trials) 177 and 180 entered the A and B canals respectively The results are consistent with the assumption that the probability for the fishes to enter one of the two canals is 0.5

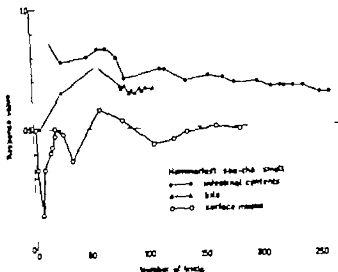


Fig 5 The response value for mature char towards smolt material intestinal contents (●), bile (▲) and surface mucus (O)

could be argued that any attractive effect of the its or secretion from the fishes might be due to l attraction caused by food remains in the gut. is hardly likely since fishes like any other nal must be expected to know the difference ween food and definite non-food In any case, racts of food pellets normally given to smolts re tested. The results show that, at one stage, the ponse value actually passed the 1% level, but in long run ended up at 0.5 The fishes used for this eriment were 2½ years of age and accepted food lets given to them. However in most experi- nts older fishes were used, and they showed no erest in food pellets. This was studied and con- med every year

Response to crud secretions from smolt Ex periments with smolts as odour donors showed that the mature sea char were attracted to substances duced by the smolts In different sessions of periments the mature sea chars were studied with pect to their reactions to secretions produced by the smolts The materials which were studied were intestinal contents skin mucus and bile from the l bladder The results of one session of tests t h of these materials are compiled in Fig. 5 Skin mucus, collected with the vacuum cleaning method (Stabell & Selset 1980) did not attract the mature char The results from one session in 1977 can be seen in the Fig 5 diagram where the response value in 34 test periods giving 182 trial is summarized. In the 1976 experiments, the mucus seemed to be at tractive but this mucus was collected by a method known to give heavy contamination with intestinal contents (Stabell & Selset 1980). The same year another mixture of mucus and intestinal contents gave no attraction This negative result remains unexplained

The intestinal contents collected by the method described by Selset (1980) elicited strong attraction responses in mature char The results from the test session with the same mature fishes as used for tests with skin mucus are shown in the Fig. 5 diagram. In one test session, the response to intestinal contents was indifferent, but the material had then been collected from starved smolts which were later confirmed as sick.

Since the intestinal contents attracted the fishes and since the attractants were suspected to be some kind of bile acid derivative (Selset 1980) a series of experiments were performed in 1978 to see if bile from the gall bladder of the smolts was attractive to the mature chars The results of these tests are shown in Fig. 5 In test period numbers 5 and 6 represented by the 2nd and 3rd triangle in the fig ure, the bile seemed to have a powerful attraction The results of these two tests brought the response value beyond the 1% level. In the following tests the fish behaved indifferent to the bile However the response value stayed above the 1% level due to the cumulative presentation of the results

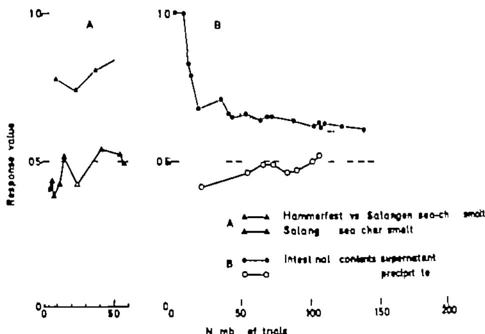


Fig 6 The response values for mature Hammerfest char (A) Towards Hammerfest smolt intestinal contents versus Salangen smolt intestinal contents (▲) and towards Salangen smolt intestinal contents alone (Δ) (B) Towards supernatant (HAS) and precipitate (HAB) of intestinal contents from Hammerfest char smolts (● and O respectively)

Responses to different char populations Whether or not the attractive substances were specific to the Hammerfest population of char was planned to be investigated thoroughly but this was prevented by new quarantine regulations at the Fish Breeding Experimental Station However competition tests were performed with intestinal contents from smolts of a different population of anadromous char from the Salangen river system Test solutions of intestinal contents from Hammerfest smolts and Salangen smolts were prepared from one fish each A preference for the sample from its own population was observed as can be seen from the diagram in Fig. 6 A This result may be suspected as partly due to a repellent effect from the strange smell Therefore the sample of Salangen material was tested alone but showed no repulsive effect (Fig 6 A) However the intestinal contents of the Salangen smolts were transported by mail to Sunndalsora and possible attractants could have been destroyed by microbial processes in the time lag

Another competition test was performed using live Hammerfest smolts and 2½ year old stationary chars (Tinnsjo Rjukan) as odour donors These tests were conducted in 1977 right after the very convincing experiments with smolts alone The results showed an almost significant (1% level) attraction of the mature Hammerfest chars to the Tr[illegible] freshwater population This puzzling result c[illegible] be explained in two ways The attraction co[illegible] caused by some kind of conditioning si[illegible] Tinnsjo chars had been kept together with the [illegible] and 1974 generations of Hammerfest chars th[illegible] 1½ years before the experiment The attra[illegible] could also possibly be explained by sex attra[illegible] since the Tinnsjo chars seemed to be ar[illegible] (beautiful spawning colours) in spite of lo[illegible] Since the smolts alone were so attractive to mature chars just before the strange popula[illegible] was judged to be at least as attractive as the sm[illegible]

Among the fractions of the intestinal con[illegible] described in the preceding article (Selset 19[illegible] convincing attraction effect was induced b[illegible] supernatant (HAS) whereas the precipitate (H[illegible] gave no response (Fig 6 B) These tests were [illegible]ried out in 1976 with a starting material that [illegible] mixture of intestinal contents and surface [illegible] Of the gel filtration fractions of this material [illegible] the one corresponding to HAS-2 was judged [illegible] attractive This fraction is among the retarded [illegible] which have been shown to originate almost ex[illegible]sively from intestinal contents (Stabell & S[illegible] 1980) Since then only intestinal contents [illegible] been fractionated further

Fig. 7. The response values for mature char towards fractions of the supernatant (HAS) of intestinal contents, HAS-1 ( ), HAS- (●) and HAS-3 (Δ).

The presence of attractive substances in the HAS- fraction were confirmed by the experiments of 1977 when HAS-1, HAS-3 and subfractions of HAS-2 were tested. The results of these experiments are presented in Fig. 7 and 8. HAS-1 could possibly turn out to be attractive, but corresponding fractions did not give attraction in the 1976 experiment.

The only subfraction of the HAS-2 judged to be attractive was HAS-2-B-3. HAS-2-B-4, the neighbouring fraction might also be considered weakly attractive. All the HAS-2-B fractions were tested at a concentration of 1.5×10^{-9} g/l.

The fractions of HAS-2 A (one through four) were pooled to be tested as one solution to save time. These fractions seemed not to elicit significant attraction in the behavioural studies as can be seen from the diagrams in Fig. 8. However, it should be kept in mind that the number of trials made in some of these behavioural tests was low due to paucity of available test material.

DISCUSSION

The result of the present experiments has shown that the anadromous char of a population from Northern Norway is attracted to the scent of smolts from its own strain. The experiments have also provided evidence that the substances are released with the intestinal contents and might possibly be found in the bile of the gall bladder. Each of these points raises a number of questions and will be discussed. The experimental test situation also requires consideration, and finally the implications of the present results in the light of the pheromone hypothesis proposed by Nordeng (1971, 1977) will be discussed.

The behavioural experiments meet with all the requirements for the binomial model, except for one. The probability for the fishes to choose a cer

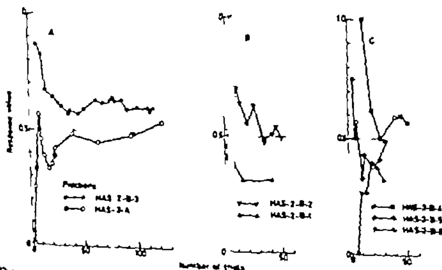


Fig. 8. The response values of mature char towards the fraction HAS-2 A and the subfractions of HAS-2-B. See text.

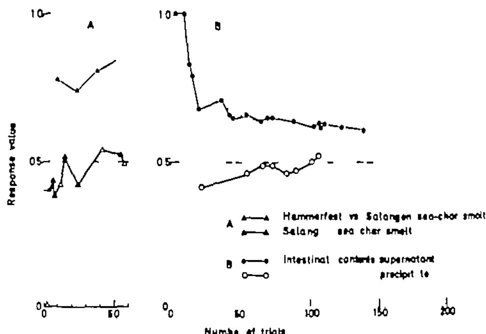


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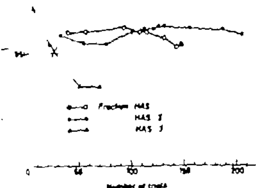


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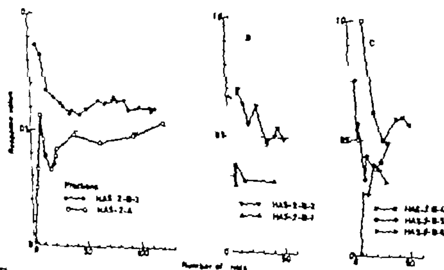


Fig. 8. The response values of mature char towards the fraction HAS-2-A and the subfractions of HAS-2-B. See text.

Table 2 *Final data for the behaviour tests*
The probability values are correct only if the binomial model is valid for the experiments

Test solution	Year tested	No of test periods	Total no of trials (*N*)	No of successful trials (*Y*)	Probability in % for *Y* or more successful trials of *N* trials
Hammerfest sea-char smolt	1977	58	313	219	<0 01
Hammerfest sea-char smolt	*1978*	*38*	*153*	9[illegible]	0.76
Hammerfest sea-char smolt					
Intestinal contents	1976	[illegible]3	[illegible]78	156	2 4
Intestinal contents	*1977*	*4*	*261*	*165*	<0 01
Intestinal contents	1978	30	155	98	0 07
Hammerfest sea-char smolt surface mucus	1977	34	183	90	56
Intestinal contents supernatant (HAS)	*1976*	[illegible]2	*218*	123	3 4
Intestinal contents precipitate (HAB)	1976	8	105	55	33
HAS-1	1977	8	150	79	28
HAS 2	1976	12	210	119	3 1
HAS-3	1977	4	71	25	99*
HAS 2 A	1978	18	123	66	4
HAS-2-B 1	1978	10	42	13	99*
HAS-2-B 2	1978	14	51	[illegible]5	50
HAS-2-B 3	1978	24	114	68	4
HAS- -B-4	1978	8	51	79	20
HAS-2-B 5	*1978*	*8*	*33*	*16*	50
HAS-2 B-6	*1978*	*8*	*31*	*10*	96
Hammerfest sea-char smolt, bile	1978	28	108	71	0 08
Food pellets	*1977*	*26*	294	148	48
Hammerfest vs Salangen sea-char smolt					
Intestinal contents	1978	4	53	45	<0 01
Salangen sea-char smolt intestinal contents	1978	10	57	28	50
Hammerfest smolt vs Tinnsjø char	*1977*	*50*	*194*	*80*	99*
Bl od test.	1976	[illegible]6	357	162	96

Indicates repellent effect of the test solution
* Indicates that the odour from the strange population is preferred

tain canal seemed to be variable Periodic preference for one of the canals was observed this preference could be for either canal and last up to several hours This could however be caused by internal social relations between the fishes in the test compartment For instance if a hierarchy is established the position of the leader fish within the system could easily have consequences for canal selection by the other fishes Therefore the ideal way of testing would be to use only one fish at a time As mentioned earlier however this was no practical way to perform the experiments with the systems used To reduce the necessary number of trials for each test solution the number of canals arranged in a symmetrical way should be extended

In some behaviour experiments it would be sufficient to let the fishes perform a certain number trials and then calculate the probability and sig cance of the final result However to conduct t experiments in this way one needs additional formation on fish behaviour For example whet the fishes will be searching for the postulat "pheromones" constantly or only at certain peri of the day In the latter case one would need ass ance that the tests were performed only at the re periods Another question is whether or not t migrating instinct will be "switched off" once t fishes have reached fresh water containing t pheromone The development of the trials of a e session is therefore given in graphs

The results of the experiments with the fractio of intestinal contents are no s evident as th

the crude intestinal material itself However total results add up to a fairly reliable anticipa- of the attractive substance(s) to be found the fraction HAS-2-B-3 This judgment was [illegible]thened by the fact that in physiological ex[illegible]ments the olfactory organ had a threshold (00 [illegible]s lower to this fraction than to any of the others [illegible]ng et al. 1979 unpublished). The threshold [illegible]surements were performed after the last series behavioural experiments. A weak behavioural [illegible]ponse to a pure attractant could be suspected [illegible] fish that are exposed to a single odorant with- the natural odorous background could become [illegible]picious and careful The local river from which water was taken contains no chars. A complete [illegible]ction of the natural response could perhaps be result A weak response to the pure attractant [illegible]ld also be expected if several substances were [illegible]olved in a synergistic way to induce full response [illegible]der natural conditions

The experiments with live smolts showed that the [illegible]ture chars are attracted to smolts from their own [illegible]pulation at the time for natural homeward migra-

The competition experiments suggest that the act[illegible]e substances might be specific for the [illegible]mmerfest population of chars The results from competition tests with Hammerfest smolts and [illegible]ore Tinnsjo chars could be evidence of an im[illegible]nting phenomenon but the explanation that the [illegible] were attracted to some sexual component is [illegible]sible

The behavioural experiments evidence that at[illegible] [illegible]ti e substances are found in the intestinal con[illegible]ts. The skin mucus has been shown to contain [illegible]rent olfactory substances (Døving et al 1973 [illegible]74) but this material might have been contami[illegible]ted with intestinal contents either directly by the [illegible]pling procedure or indirectly since these sub[illegible]nces [illegible] be present in the water and might be [illegible]sorbed by the skin mucu The skin mucus has [illegible]en suggested to be the source of the "migration [illegible]eromones" (Nordeng 1971), but the present ex[illegible]riments suggest other sources However "pure [illegible] in mucus was only tested once and cannot there[illegible]re be totally excluded [illegible] source of attractants. [illegible] addition the migration pheromones may not [illegible]nction in the artificial situation of the behaviour [illegible] periments.

[illegible] If the attractant are found in the bile, as the [illegible]periments indicate the chemical isolation of the [illegible]bstances would be greatly simplified. This would also mean that these substances are likely to be synthesized in the liver However there is still the possibility that the attractants are produced by in-testinal micro-organisms and returned to the bile via the entero-hepatic circulation

The results of the present study support the pheromone hypothesis presented by Nordeng (1971 1977) which explains the migration of sal-monids from their hatching areas in the river system out to sea and back to their home spawning grounds. His observations and experimental find-ings can most reasonably be explained by the pres-ence of substances from the smolts of the same population released into the water to form the trail by which first the mature fish, and then the smolts, can follow back to the spawning grounds.

Our results however do not exclude an olfactory imprinting but make it likely that the possible "imprinting would be to substances emanating from the fishes themselves and not from vegetation or minerals (Hasler 1966) An optimal way to eluci-date the mechanism of salmonid migration could be to identify the guiding substances and their natural source and thus to enable imprinting experi-ments with the natural group of substances

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·factory sensitivity to bile acids in ımonid fishes

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DØVING K B SELSET R. & THOMMESEN G Olfactory sensitivity to bile acids in salmonid fishes. Acta Physiol Scand 1980, 108: 123–131 Received 24 April 1979
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Monopolar DC-recordings were made simultaneously from two positions on the olfactory bulb of chars (Salmo alpinus L.) and graylings (Thymallus thymallus L.) using bile acids and amino acids as olfactory stimulants The bile acids induced responses with characteristic spatial differences from those of the amino acids. The distribution of responses to bile acids indicated a neuronal activity in the medial part of the bulb. In contrast, amino acid elicit responses in the lateral part of the bulb Taurine conjugated bile acids were up to 1 000 times more potent as olfactory stimuli than methionine. The results suggest that olfactory receptors are of two types, one responding to bile acids the other to amino acids. 3-α-hydroxysteroids are released from the fish into the water in quantities that suffice for detection by their olfactory system. The odorant potency of the bile acids their evolutionary history and variability together with their renowned adherent properties made them interesting candidates for specific signals in the aquatic environment.

Key ords Fish, olfactory bulb, induced potentials spatial distribution, odour potency bile bile salts amino acids, chemical signals, migration

accurate homing of salmonids has been shown ave an olfactory basis (Wisby & Hasler 1954 es et al. 1968 Toft 1975). Odorous substances can provide such an accurate migration over distances and time must have properties that be described in terms of diversity specificity ncy and stability The theory proposed by deng in 1971 for the mechanism of homing by nonid fishes requires that the odorous subces emanate from fishes in the river system, from smolts on their seaward migration.

hysiological experiments ha e shown that fishes luce substances that are potent stimuli to the ctory organ (Døving et al 1973) and that there ts a neuronal basis for discrimination of differ strains of fishes (Døving et al. 1974). By recordfrom the bulbar surface of salmonids, Thomme (1978) showed that amino acids used as odorgave responses in the lateral part of the bulb n water containing fish elicited responses in the ral and the medial part of the bulb At that time chemical nature of the substances that elicited onses in the medial parts were unknown.

In the present study we show that bile acids evoke responses in the medial part of the bulb The majority of pure bile acids tested, elicited distinct responses in concentrations below the ones usually considered to be thresholds for amino acids The possible function as odorants of this group of chemical compounds is discussed. The results of the present study have been presented previously as a short communication (Døving, Selset & Thommesen 1978)

MATERIAL AND METHODS

The experimental fishes used in the present study were anadromous char Salmo alpinus (L.) syn. Salvelinus alpinus (L.) and grayling Thymallus thymallus (L.). The chars were raised at the Fish Breeding Experimental Station, Sunndalsøra, Western Norway but originated from the Hammerfest region of Northern Norway The graylings were caught in small creeks of Lake Mjøsa in Southern Norway The weights of the fishes were between 200 and 900 g. The fishes were transferred to the aquarium facilities at the University of Oslo before use In the experiments the fishes were immobilized with d-tubocurarine

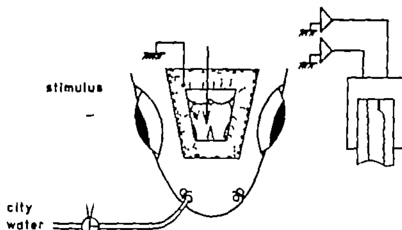


Fig 1 Schematical diagram of the stimulation and recording arrangements. The relative size of olfactory bulbs is exaggerated

(Nyegaard & Co Oslo) 10 mg/kg i m. The fish was wrapped in a wet sponge and maintained in a fish holder under continuous flushing of the gills with tap water at 5–8°C The dorsal part of the brain case was opened and the olfactory bulbs exposed (Fig 1) During the surgical procedure the fish was usually anaesthetized with MS 222 (Sandoz) by infusion into the respiratory water flow to a final concentration of 100 ppm/(mg/l) Exceptions were fishes serving as controls

Recordings The potentials were picked up by use of glass capillaries filled with a physiological salt solution The electrodes were inserted into electrode holders containing sintered Ag/AgCl Electrode tip diameter ranged from 20–100 μm. The electrode tips were placed on the dorsal surface of the olfactory bulb The potential changes were recorded using preamplifiers with frequency response DC 70 Hz and monitored on a pen recorder All recordings were monopolar and the preparation was grounded through the indifferent electrode only which was placed on the cranium just posterior to the opening in the skull (Fig 1)

Stimulation The stimuli were introduced from a polyethylene pipette through a continuous flow of aquarium water into the ipsilateral anterior naris and controlled by a 3-way stopcock Nasal water flow was kept at 0 3 ml/s.

Fish basin water samples bile from both species and the 11 substances listed in Table 1 were used as stimuli

The chemical composition of bile acid derivatives is by the drawing of cholanic acid in Fig and the p of the added groups listed in Table 1 All stimuli diluted in decadic steps the pure substances from to 10^{-10} M the bile in dilutions from 4×10 to vol/vol

Experimental procedure Responses have been recorded from both olfactory bulbs stimulating the ipsilateral olfactory epithelium The recording electrodes placed in different positions on the dorsal surface of bulb Responses from a medial and a lateral position recorded simultaneously in each experiment.

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Each kind of stimulus was presented in the order of ascending concentration each concentration tested twice consecutively The order of the stimuli varied at random To avoid fatigue stimulation intervals were at least 30 s or as long as any sign of the previous response remained Stimulation time was marked ly and later adjusted for the time lag present due to construction of the stimulation device Each preparation was initially checked by testing the response to methionine sulfotaurolithocholic acid and a water sample from the fish basin

Evaluation of responses In principle evaluation of magnitude of the bulb surface response involve

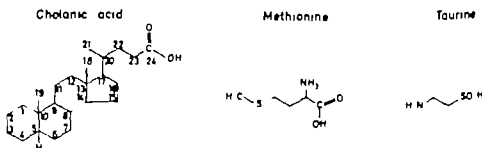


Fig 2 Chemical structure of the stimulating substances For the position of substituents on the cholanic acid skeleton see Table 1

1 *Threshold values of pure compounds*

...ecture compare with Fig. 2

...on name	Position of groups OH	SO$_3$H	Tau	Mean threshold value (M)	Threshold value range (M)
...acid	3 7 12			8.0 $10^{-[illegible]}$	$10^{[illegible]}$ $10^{-[illegible]}$
...cholic	3 7 12		24	2.0×$10^{-[illegible]}$	$10^{-[illegible]}$–$10^{-[illegible]}$
...cholic	3 12				–
...deoxy- acid	3 12		24	6.3×$10^{-[illegible]}$	$10^{-[illegible]}$–$10^{-[illegible]}$
...deoxy acid	3 7				–
...chenodeoxy- acid	3 7		24	4.0×$10^{-[illegible]}$	$10^{-[illegible]}$–$10^{-[illegible]}$
...cholic	3				
...litho- ...ic acid	3		24	6.3×$10^{-[illegible]}$	$10^{-[illegible]}$–$10^{-[illegible]}$
...tauro- ...cholic		3	4	1.0 $10^{-[illegible]}$	$10^{-[illegible]}$ $10^{[illegible]}$
...somine				1.3×$10^{-[illegible]}$	$10^{-[illegible]}$ $10^{-[illegible]}$
...ne				1.0×$10^{-[illegible]}$	

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...urement of DC shifts and AC amplitudes independ... However for each type of stimulus the sum of these ...use components are proportional as long as the ...ding positions are maintained (Thommesen 1978). In ...present work we have therefore concentrated on the ...deflection as being the more rapidly measured para... ...t.

...e DC potential shifts were measured as the mean ...ction of the tracing at 3–4 ... after the onset of the ...ulse. Due to the general instability of the DC record... ...he measurements of the DC deflections are inaccurate ... 50 µV.

...ULTS

...eported in previous investigations, the electri... ...ctivity recorded from the surface of the olfac... ...bulb in fishes exhibits irregular and spontane... fluctuations (Døving et al. 1973, Døving & Belg... ...1977, Thommesen 1978) comparable to those ...d in the frog (Ottoson 1959b). Stimulation with ...r containing an odorant induced changes in the ...earance of the electrical activity. These re... ...ses of the olfactory bulb consists of a slow ...ate potential upon which rhythmic oscillations ...nduced waves are superimposed (Adrian 1950, ...son 1959a, b). Some examples of these record... ...are seen in Fig. 3. To prepare these record... ...the dorsal surface of the olfactory bulb was searched to find the areas most sensitive to sulfotaurolithocholic acid and methionine respectively. The recordings shown in Fig. 3 are taken from a series of experiments where the responses at these positions of the bulb were investigated with different concentrations. As seen, there is a slight change in DC potential of the recording trace when stimulating with sulfotaurolithocholic acid at a concentration of 10^{-12} M, clear induced waves are not seen. When stimulating with a concentration 10 times higher there was a distinct DC potential shift together with clear indications of induced waves. Whenever found the induced waves appeared at the same concentration step where a significant DC potential shift was seen.

Concentrations. The results shown in Fig. 3 are typical of the responses obtained in areas of the olfactory bulb reacting to each of the two substances. The amplitude of the DC shift increased with the concentrations as did the amplitude of the induced waves. The maximum amplitudes of the DC shifts were about 1 mV, the induced waves could be in the order of 0.4 mV peak to peak at most. The variations of the AC and DC responses in relation to the concentrations have been described by Thommesen (1978). These results indicated that the neuronal pool activated by the odorous stimuli

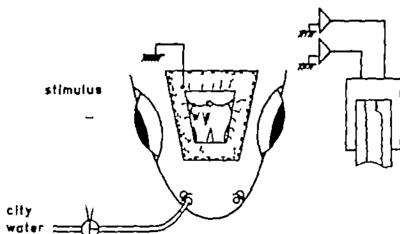


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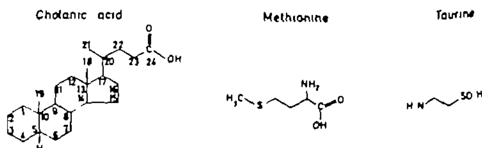


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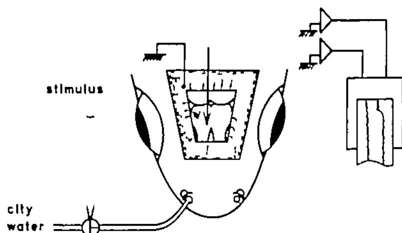


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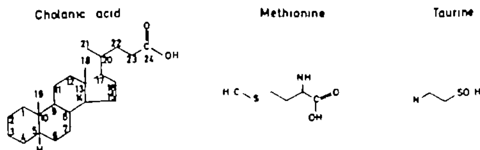
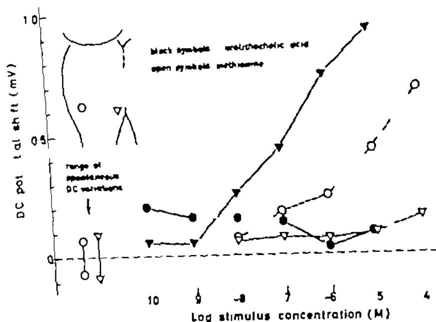


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f Diagram of the relation between the bulbar responses and stimulus strength. The magnitude of DC potential shifts sponses to methionine and taurolithocholic acid are plotted against the different concentrations

nses occurred between methionine and tauro- lic acid.

hemical structure and esponse threshold The erent unconjugated bile acids were not as potent odorants as the conjugated ones. The mean sen- vity for the different compounds are given in le 1. For comparison the table includes the lts for methionine and taurine as well. The ine conjugates and especially the sulfonated one regularly a lower threshold for eliciting re- nses in the olfactory system than any of the onjugated derivatives. The absolute sensitivity ied for the different preparations, but the rank followed nearly the same order in all experi- nt. Some of the bile acids derivatives have so solubilities that their thresholds could not be sfactorily determined. These were therefore luded from further investigations. These sub- ces are marked with an in Table 1.

atural products Crude bile taken from the bladder of the two species used in the present periments was tested. Responses were obtained dilutions down to about 1:10⁶. Distribution of ponses to the bile was similar to that of the pure e acids.

Basin water containing fish gave responses both in the medial part of the olfactory bulb, i.e. at the same area as the bile acids, and in the lateral part (Thommesen 1978). The presence of bile acids in basin water was investigated by holding a char in 10 l of water for 4 h. The water was then analyzed for its contents of 3-α-hydroxysteroids in relation to a control (Sterognost 3-α, Nyegaard et Co., Oslo). The analysis showed an effluence of 3-OH steroids of 34 nmol kg h. This result indicates that a sufficient quantity of bile acids emanate from the fish to be detectable by the olfactory system of the fish. Artificial mixtures of methionine and bile in different concentrations elicited responses that were qualitatively and quantitatively intermediary between the responses to each of the constituents. It was thus possible to immitate responses to basin water by an artifical mixture of known substances.

Most of the present experiments were performed in May and June 1978 with pilot studies in April and some supplementary tests in August. Late in August, the fishes gradually lost their sensitivity to all olfactory stimuli tried. In January 1979, the fishes regained their sensitivity to basin water samples and to some extent to diluted bile, to a lesser extent to the pure substances. The reason for this change in sensitivity is unknown.

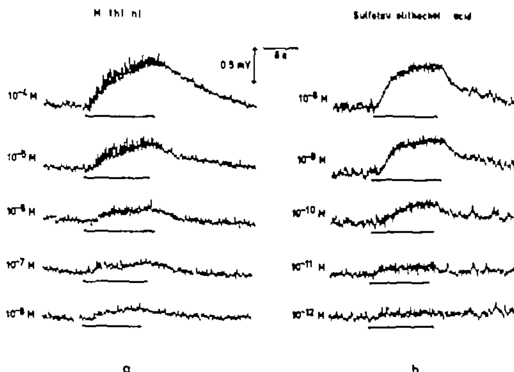


Fig 3 Responses to different concentrations of methionine and sulfotaurolithocholic acid obtained at their r recording positions Note the different concentration levels in the two series.

increased and/or that the depolarization of the indi vidual neurones and syncronizations of spike activity increased with increasing stimulus concentration

An example of variation in the amplitude of these responses with concentration of the stimulus can be seen in Fig 4 The electrode positions are in the anterior region one lateral and the other medial As seen the medial part of the bulb responded only to the taurolithocholic acid The lateral part of the bulb responded to methionine Responses increased approximately linearly with the logarithm of the stimulus concentration

Spatial distribution of responses As demon strated by studies by Thommesen (1978) the responses recorded from the olfactory bulb depend on the electrode positions This dependence also appears from the results shown in Fig 4 Two of the recordings from the experimental series making up Fig 4 are displayed in facsimile in Fig. 5 By record ing simultaneously from two regions of the dorsal surface of the olfactory bulb as indicated in the schematic drawing of Fig. 5 the differential sen sitivity of the regions is clearly demonstrated The upper part shows recordings during stimulation with taurolithocholic acid at a concentration of 10^{-7} M while the lower part shows the responses to stimulation with 10^{-4} M of methionine Taurolitho-cholic acid gave a large response in the medial of the bulb but hardly any response in the l part In contrast methionine gave a consider response in the lateral region and hardly any r medial part of the bulb

Further information of the spatial distribution the responses was obtained by exploring the b surface with two electrodes and studying the sponses to a concentration series of a potent acid and methionine As response parameter measured the magnitude of the DC potential r confirming the distribution of the response. considering the magnitude and polarity of the duced waves

Cross-adaptation The spatial isolation of bulbar responses to the two types of st suggests that there are two groups of olfactory ceptors responding either to amino acids or to acids As pilot experiments for receptor st cross adaptation experiments were made 10^{-4} M of methionine and 10 M of taurocholic acid The bulbar responses were recorded switching directly from one stimulating substance to the other Even after 11 min of continuous exposure to one of these stimuli the immediate sponse to the other was unaltered Thus appa ly no significant cross-a n ion of the bulbar

·s with amino acids elicited responses in the ·al part of the olfactory bulb whereas crude fish ·r gave responses in both lateral and medial

The results of the present experiments dem·trate that some bile acids elicit responses in medial part of the bulb only

·ceptors The spatial separation of the re·nses to representatives of the amino acid and the ·acid groups, suggests that the acceptors for the ·kinds of stimuli should be located in separate ·ptor cells Preliminary experiments indicate a ·of cross adaptation between representatives of ·e two groups of compounds thus they pre·ably activate discrete pathways in the olfactory ·tem One type of receptor cell should therefore ·y acceptors for the bile acids, another for amino ·ls I the goldfish there are two morphological ·es of receptor cells, one with microvilli and ·ther w th cilia (Ichikawa & Ueda 1977) The ·e morphological cell types have been found in ·pacific salmon (Yamamoto & Ueda 1977) pre·nably with the same function. It is an interesting ·sibility that each morphological type has accep· ·responding to one of the two kinds of chemical ·muli

Receptor distribution Since the olfactory bulb ·s as a relay station between the peripheral ·ithelium and the central nervous system our find·gs on the spatial distribution of the responses in ·e olfactory bulb have implications both for the ·nvergence of receptors and the projection of sec·ndary neurones from the bulb Theoretically the ·o different kinds of receptor cells, those respond·g to amino acid and the other responding to bile ·ids, might either be topographically separated or ·ermingled in the olfactory epithelium. A topo·aphically separated distribution seems more ·ely when considering the spatial distribution of ·e responses at the level of the olfactory bulb. An ·termingled distribution might be of advantage ·ith respect to the water sampling of the olfactory ·gan.

·Cent al projections The secondary neurones of ·e olfactory bulb project to the central nervous ·ystem via distinct bundles that form the olfactory ·act (Sheldon 1912, Døving & Gemne 1965) The ·rojection is arranged so that neurones mainly ·tuated in the lateral part of the bulb constitute the ·teral tract and neurones mainly in the medial part ·rm the medial tract (Døving et al in preparation). ·his subdivision of the olfactory tract strongly indi·cates that the bundles serve different functions. The results of the present investigation imply that these functions may be connected to different groups of chemicals.

Bile acid In salmonids the bile acids produced are chiefly the taurine conjugates of cholic and chenodeoxycholic acid (Nagayoshi et al 1964 Sasaki 1966, Haslewood 1967 Ripatti et al 1973 Denton et al. 1974) What happens to the bile as it descends the intestine in fishes is largely unknown, but our experiments have shown that anadromous char release about 34 nmol kg^{-1} h of 3-α-hydroxysteroids into the surrounding water

The threshold for eliciting bulbar responses to basin water containing *fish of the same population* is in the range of 0.03 fish h^{-1} l (Thommesen, unpublished results) Each fish weighs about 0.2 kg. Thus the threshold is equal to 0 006 kg h l

When considering the production rate of 34 nmol h kg the threshold for the fish olfactory system to detect these substances will be about 2×10^{-10} M and thus at the same concentration level as the threshold for the more potent of the bile acids. This calculation shows that the production of 3-OH bile acids could account for the bile acid like part of the responses to basin water but does not exclude the existence of other potent odorants

The olfactory sensitivity of the fishes was shown to vary reaching a minimum during the autumn A similar loss of sensitivity has been observed in another population of char during the early part of the winter (Thommesen 1978) One cause for this variation may be a natural change in olfactory sensitivity but a poisoning action of the aquarial water supply could be a possibility It is most reasonable that this decrease of olfactory sensitivity reflects an annual change that follows the life cycle of these fishes Whatever the cause may be, this selective anosmia to the pure amino acids and bile acids tested compared with a recovery of the sensitivity to basin water indicates that the basin water may contain potent odorants which are different from the pure substances tested so far Thus we are dealing with either a third class of odorant receptors in the epithelium and/or annual changes in olfactory sensitivity t specific substances.

The evolutionary history of the bile acids i ver tebrates has been reviewed by Haslewood (1967) He summarized the vast amount of findings of the bile salt chemistry by pointing out that there has been progression from $C_{27}5\alpha$ alcohol sulfates to

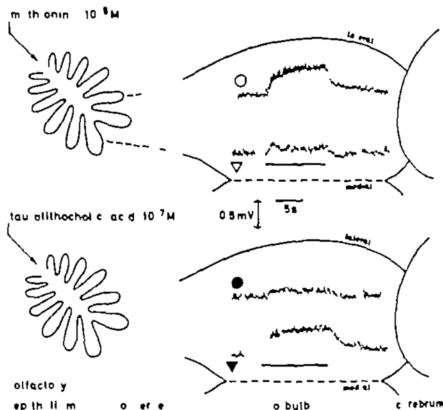


Fig. 5. The appearance of simultaneous recordings from two positions on the salmonid olfactory bulb during stimulation with an amino acid versus a bile acid.

DISCUSSION

The results of the present series of experiments show that some bile acids, especially taurine and sulfate conjugates, are potent olfactory stimuli for salmonid fishes. Previous investigations have shown that the olfactory organ of fishes is sensitive to amino acids (Sutterlin & Sutterlin 1971, Suzuki & Tucker 1971, Belghaug & Døving 1977, Hara 1973, 1976, Caprio 1978). In our present study, we demonstrate that the fish olfactory system is more sensitive to some of the bile acids than it is to amino acids. Responses to the bile acids in the olfactory bulb are spatially separated from those to the amino acids, thus the results have implications for our understanding of the discriminatory mechanisms in the fish olfactory system. Bile acids and their derivatives have chemical properties that render them interesting as mediators of information in the aquatic environment. The possibility that these substances might be important for many functions in the life span of fishes, especially in the homeward migration of salmonids, is of particular concern. In the following, different aspects of the function of the fish olfactory system will be considered in view of this new information.

Sensitivity. The amino acids have previ... been considered to be the most potent grou... olfactory stimuli for fish. In this study, using ... acids as stimuli, responses were obtained fro... olfactory bulb surface at much lower concen... tions than ever observed when using amino a... The bile acid derivatives are therefore presum... more potent as olfactory stimuli than the a... acids or any other group of substances examine... electrophysiological techniques (Hara 1976, B... haug & Døving 1977).

The bile acids may be suspected of having ... ally irritating effects on receptor membranes ... cause of their strong detergent properties. H... ever, the substances did not evoke activity in ... areas responding to amino acids as they would h... done if their effect had been a general "stimulati... of all receptors. This high sensitivity to the b... acids can only be explained as a specific sensi... to this particular molecular configuration. T... the olfactory receptors there must be accept... specifically designed to react to the bile acid der... tives.

Spatial distribution. Thommesen (1978) sho... that stimulation of the ol... organ of salm...

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$C_{24}5\beta$ acids There are unique stages in the vertebrate evolution that might be considered representative for the different vertebrate groups Thus the bile salt history could indicate that a concomitant sensitivity to these chemical substances has a survival value The known variability of bile salts and the multitude of their derivatives of which only a minor part is known yield interesting perspectives for chemical communication among fishes We demonstrate in the present experiments that the fish olfactory system is sensitive to some of these compounds that it is similarly sensitive to bile per se and that there are substances of bile salt character in the water inducing olfactory response in the same areas of the bulb as the pure bile compounds These observations all indicate that the bile and bile derivatives might be valuable sources of information for fishes

Bile salts properties The principle function of the bile salts in the vertebrate body is to aid in the digestion of food lipids by their detergent properties Their chemical properties make them particulary interesting in the aquatic environment because some of them are readily degraded and would have only a short term effect on a fish Others are more stable and are absorbed by organic matter and minerals in the water This could give the fishes a possibility for recognizing their own territory

Homing and olfaction The homing of salmonid fishes has been shown to depend upon the intact function of the olfactory organ as for example demonstrated by Wisby & Hasler (1954) and Groves et al (1968) Nordeng (1971 1977) has on the basis of extensive field observations proposed that anadromous salmonids follow odour trails which are produced by other individuals of the same population in the water system To secure a successful homing based on olfactory cues the threshold of the olfactory system of each individual migrating fish needs to be lower than the concentration of odorants emanating from the fish population in the water system This reasonable assumption can be formulated to yield a general expression of the possibility of fishes to home securely by olfactory cues The ratio between the concentration of fish odorants in the water system and the odour threshold in homing fish expresses a safety factor for homing The concentration will depend upon the number of fishes in the river the production of odorous substances from each individual fish and the flow of water through the system

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ect of a pressure barrier on retrograde axoplasmic transport in vitro A study in the motor neurons the rabbit vagus

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HAHNENBERGER, R. W. Effect of a pressure barrier on retrograde axoplasmic transport in vitro A study in the motor neurons of the rabbit vagus. Acta Physiol Scand 1980, 108: 133–137 Received 26 Feb. 1979 ISSN 0001-6772. Departments of Medical Pharmacology and Ophthalmology University of Uppsala, Sweden.

Retrograde axoplasmic transport, demonstrated by the "double ligature method" was studied in vitro at the motor neurons of rabbit vagus nerve, labelled with ^{3}H-leucine When pressure barrier with maximal pressure of 30 mmHg, induced by fluid jet, was applied to small section of the nerve at 38°C, retrograde flow was partially inhibited. More radioactivity was found in front of the barrier (with respect to direction of flow) than immediately behind it. This observation differs from findings in anterograde transport at the same pressure and tested with similar technique where most radioactivity was detected immediately behind the barrier (Hahnenberger 1978).

Key words Rabbits, vagus nerve retrograde axoplasmic transport, pressure barrier

rograde axoplasmic flow by which material is sported towards the perikaryon, can be inhib by ischemia (Kristensson et al 1971) and var agents such as iodoacetate 2,4-dinitrophenol ide (Edstrom & Hansson 1973) and antimito- (McLean et al 1976, Edstrom & Hansson 1973 t 1972), indicating a close similarity to antero- le transport. However whereas anterograde occurs in vivo with at least two distinctly dif nt rates, a fast (10–100 mm/h) and a slow (1–2 /day) (Dahlström 1971), there is up till now no lence for a retrograde transport rate as slow rograde. Retrograde transport is approximately as fast as forward flow (Kristensson et al 1971) seems to be more sensitive to colchicine Lean et al 1976).

n a previous paper the effect of a pressure bar on fast anterograde axoplasmic flow in the or neuron of the rabbit vagus nerve was re ted (Hahnenberger 1978). The purpose of the sent study was to investigate the effect of pres- e on retrograde transport, utilizing the same niques for local compression of the nerve

MATERIALS AND METHODS

Animals and anesthesia

Albino rabbits of both sexes weighing between 1.8 and 4.7 kg were used. Anesthesia was obtained by slow i.v. injection of 7–8 ml/kg of 25% urethane solution (w/v). Occasionally ether was added, when surgical anesthesia was not achieved with urethane alone.

Labelling of the motor neurons of the vagus nerv

40 μCi ^{3}H-leucine was applied in drops to the floor of the 4 ventricle After 3.5 h the animal was killed and 2 ligatures applied to the vagus on each side: one proximal (L_p), rostral to the ganglion nodosum and one distal (L_d) 50 to 60 mm from L_p. For details of the procedure see Hahnenberger (1978).

Pressure barrier

The same method as described previously was used. The nerve was mounted in superfusion chamber and aerated superfusion solution (composition see Hahnenberger 1978) was directed through an annular gap around the whole circumference of small section of the nerve Gap width was 0.2 mm. A flow rate of 290 ml/min resulted in a maximal pressure of 30 mmHg and maximal pressure gradient of 100 mmHg/mm nerve centered around the gap. This pressure barrier had been found to be not far above

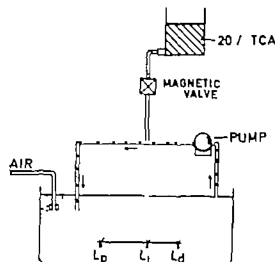


Fig 1 Arrangement for automatic interruption of axoplasmic flow

threshold for inhibition of fast anterograde axoplasmic flow at 22°C (Hahnenberger 1978) The present experiments were run at 38°C Only one nerve of each animal was used in these experiments the other nerve was used for other purposes

Retrograde axoplasmic flow

A ligature blocks all axoplasmic transport Anterogradely transported material soon accumulates at the ligature but gradually some is removed (reflected) by retrograde flow away from the ligature (Bray et al 1971 Edström & Hansson 1973 Frizell 1974) A new ligature applied proximal to the first one after the anterograde flow has passed will then eventually collect the retrogradely moving fraction This fraction contains components which are reflected at the distal ligature and others which have changed direction of movement en route This technique was first described by Bray et al (1971)

After the vagus nerve was removed from the animal the ligatures L_p and L_d applied and the nerve cleaned It was either incubated as described by McLean et al (1975) in tissue culture medium 199 in an oxygen atmosphere or incubated in aerated superfusion solution as shown in Fig 1 In both cases for 5 h at 38°C Pilot studies showed that this length of time and temperature were sufficient for most of the radioactivity to accumulate in the 2 mm nerve pieces immediately proximal to L_d There was no difference in distributions of radioactivity between nerves incubated by one or the other procedure

After the nerves were removed from incubation a third ligature (L_i) was placed 10 mm proximal from L_d Most of the radioactivity subsequently collecting in the 2 mm nerve piece immediately distal to L_i thus had arrived by retrograde movement. In order to select a duration and temperature giving easily measurable radioactivities at L_i the following experiments were carried out nerves which had been incubated for 5 h at 38°C and with L_i in place were incubated for another 5 or 1 h at 22° or 38°C

Occasionally it was unavoidable that particular incubation experiments were scheduled to end at an inconvenient time of night The incubation was then interrupted by means of the system shown in Fig 1 [illegible] were placed in a bath containing 100 ml superfusion [illegible] which was circulated vigorously by a roller pump [illegible] son Marlow) and bubbled with air Above the [illegible] reservoir containing 100 ml of 20% trichloroacetic solution (TCA). The reservoir was connected to the [illegible] through a magnetic valve which was closed during incubation At the given time 5 or 12 h after the [illegible] the incubation the pump but not the air bubbling [illegible] switched off and the magnetic valve of the TCA [illegible] opened This stopped any further axoplasmic [illegible] Some hours later the nerves were cut into [illegible] kept for at least 4 h in ice cold 10% TCA solution [illegible] TCA insoluble radioactivity was determined according to standard methods The results showed that at 38°C [illegible] h there was sufficient radioactivity at L_i (see [illegible]) Experiments where nerves were subjected to a [illegible] barrier were thus carried out at 38°C run for 1 h [illegible] not automatically discontinued The center of the [illegible] placed approximately midway between L_i and L_d [illegible] end of the superfusion the nerve was cut in the [illegible] the pressure area. The nerve segments were then [illegible] further in 2 mm pieces under the preparation [illegible] and the TCA insoluble radioactivity determined

Presentation of the data

Only the radioactivity in the distal nerve segment [illegible] was considered to have significance for the present [illegible] and this was measured

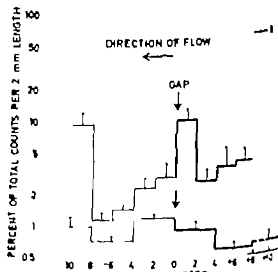


Fig [illegible] Distribution of radioactivity when retrograde (tested at 38°C for 12 h) or anterograde flow (at 22°C [illegible] h) have to surmount pressure barrier of 10 mm [illegible] upper graph represents retrograde the lower one [illegible] grade transport The latter graph has been arbitrarily moved downwards and has been constructed from data of Hahnenberger (1978 Fig 7B) Direction of [illegible] from right to left for both graphs A [illegible] significant difference between columns ($P < 0.05$) [illegible] the radioactivity is given per [illegible] mm in case of retrograde and 4 mm in case of anterograde flow Bars ind[illegible] S E M

e 1 *Distribution of radioactivity in the distal nerv segment* (L_1–L_4)

'igures represent mean percentages ± S.E.M.

ition of incubation perfusion	L_1 (2 mm)	Intermediate (16 mm)	L_4 (2 mm)	Total amount of TCA-precipitable radioactivity (cpm, mean ± S.E.M.)
ing values, 0 h (5)	3.71±0.76	19.3±3.1	77.7±2.4	5 768±685
22°C (5)	6.7±2.2	18.3±6.6	75.4 8.2	5 123±427
22°C (8)	10.4±2.6**	11.9_4.3	78.1±6.8	4 738_571
38°C (n=6)	10.6±3.0*	23.5±6.8	65.4±6.5	4983±519
38°C (=6)	18.2±3.5**	34.2±7.9*	47.7±7.9*	5 002±641
sure 30 mmHg, 12 h, C (10)	9.3±2.7	26.5±5.1	64.2±5.8	4 995±713

ficance of differences against the starting values: $P<0.05$ $^{**}P<0.025$ $^{***}P<0.0125$

he activity in each nerve piece (2 mm) between L_1 and is expressed as percentage of the whole amount cted between L_1 and L_4 (the values are given in Fig Table 1)

he two-sample t-test or the paired t-test (when suita- were used to test the significance of differences.

he expressions "proximal" and "distal" refer to the tomical course of the vagus nerve. The direction of ograde flow is disto-proximal.

RESULTS

ble 1 shows the distribution of radioactivity with- the nerve segment L_1–L_4 after L_4 was applied (0 and after subsequent incubation for 5 and 12 h at °C and 38°C

The pool of radioactivity at L_4 did not differ from initial situation (0 h) when the nerves were ubated at 22°C There was, however an increase accumulation at L_1 though only the 12 h values fered significantly from the initial values.

At 38°C the radioactivity at L_4 decreased signifi- ntly with time There was a simultaneous in- ase at L_1.

The column designated intermediate sum- arizes the radioactivity detected in the 16 mm rve segment between L_1 and L_4 (16 mm = 20 mm nus the two 2 mm nerve pieces, adjacent to both atures). This radioactivity represents mainly aterial travelling between the ligatures. At 38°C d an incubation time of 12 h there was significant y more radioactivity in the intermediate nerve gment than initially and after 12 h at 22°C <0.05).

Fig. (upper graph) shows the distribution of dioactivity after the nerve had been superfused r 12 h at 38°C A pressure barrier was applied midway between L_4 and L_1 Approximately 13% of the whole amount was found within ±2 mm of the pressure area. In the 2 mm nerve pieces immediately distal to the center of the gap (in front of the gap with respect to the direction of retrograde flow) there was significantly more radioactivity than in the 2 mm immediately proximal to the centre of the gap ($P<0.05$) (behind it with respect to the direction of retrograde flow). The significance was tested by the paired two-tailed t-test.

About 9% of the whole radioactivity in the distal nerve segment was found at L_1 This amount is significantly less ($P<0.05$) than the radioactivity collecting at L_1 when the nerves were run without a pressure barrier for 12 h at 38°C (18.2±3.5).

DISCUSSION

Retrograde axoplasmic flow can be demonstrated by two different methods. Kristensson & Olsson (1971) and later La Vail (1972) introduced the already classical "protein tracer technique" in which the uptake and retrograde movement of exogenous proteins (e g horse radish peroxidase) applied to the periphery of axons is followed. Though this method furnishes an excellent tool which does not require damage of the neuron it is at present a semiquantitative method. The "double ligature method" on the other hand provides a good quantitative resolution. However as pointed out by Frizell (1974) part of the retrograde flow could be caused by the axonal injury at the ligature rather than representing "normal" retrograde transport. Although one cannot unreservedly regard the retrograde flow studied in the present experiments as physiological, it is undoubtedly due to an active

temperature dependent retrograde transport sys tem

Though the situation in the distal nerve segment (L_r–L_4) is too complicated to interpret completely there is one point that merits discussion Nerves incubated for 5 and 12 h at 22° and 38°C without a pressure barrier showed the expected increase of radioactivity at L_1 representing retrogradely mov ing material However only when nerves were run at 38°C was there a considerable decrease of radioactivity at L_4 with simultaneous increase at L_1 and in the intermediate nerve segment This last activity may represent a slower phase of retrograde transport the existence of which has been suggested by McLean et al (1976) but the observation may also be due to a temperature dependent delay of reversal of fast moving components at L_4 The mat ter cannot be decided at present

The maximal pressue of 30 mmHg in the barrier used in the present experiments was chosen for two reasons 30 mmHg is not too far above the threshold for inhibition of fast anterograde transport and 30 mmHg has obvious relevance in connection with chronic simple glaucoma The present results show that also retrograde axoplasmic flow is clearly in hibited by a pressure of 30 mmHg It is difficult to judge however if the pressure sensitivity really differs from that of anterograde transport since the two forms of axoplasmic transport were tested in differently designed experiments Anterograde flow was tested at 22°C within a nerve segment of 50 to 60 mm and run 17 h Retrograde transport on the other hand was studied in a 20 mm nerve segment for 12 h at 38°C In addition the anterograde axo- plasmic flow studied was almost exclusively con fined to the fastest travelling components while the retrograde flow was less clearly defined and may consist of different components and rates Moreover at the starting point when the pressure barrier was applied there was presumably no radioactivity within the pressure area when an terograde flow was tested This was different in case of retrograde flow where the compression was applied to a nerve segment already containing radioactivity

The possibility that retrograde flow is more vul nerable is not disproved however and can derive some support from the findings of McLean et al (1976) who showed retrograde flow to be more sensitive to colchicine

In Fig 2 the radioactivity in the nerve pieces (4 mm each) around the centre of the gap for re grade flow is plotted in the lower graph, to be pared with the distribution of radioactivity retrograde flow was tested (upper graph) reasons of clarity both the directions of flow shown from right to left and the lower graph arbitrarily displaced downwards The figure the characteristic difference between the [illegible] of anterograde and retrograde flows While anterogradely transported material accum immediately *behind* the centre of the gap (which is also *supposedly the centre of the* pr maximum) retrograde accumulation is imm mediately *in front* of the gap The results of retrograde flow inhibition are as might be expe there is stasis of transported material in front of obstruction In contrast, the distribution of ra activity in the case of anterograde transport is difficult to understand It seems possible that accumulation in the nerve piece immediately be the gap is an accumulation of retrograde flow terial may have passed the hindrance in a for direction and then reversed its flow en route rriving retrogradely at the barrier This explana (Hahnenberger 1978) gets support from the pre data that retrograde transport is indeed accu lated in front of the impediment But whether explanation holds quantitatively remains to tested

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ntrol of tissue environment during vital croscopy of the microcirculation in the tenuissimus in cat

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AMUNDSON, B., BAGGE, U. & HALJAMÄE, H. Control of tissue environment during vital microscopy of the microcirculation in the m. tenuissimus in cat. Acta Physiol Scand 1980, 108, 139–146. Received 11 April 1979. ISSN 0001-6772. Departments of Anatomy (Laboratory of Experimental Biology), Histology and Anesthesiology, University of Göteborg, Sweden.

The physiological preservation of the tenuissimus muscle preparation in cat during vital microscopy of the microcirculation is assessed, comparing the originally described technique with a modified approach. Differences in the compared techniques include modes of dissection and transillumination, room-air exposure and monitoring procedures. The original technique involves extensive dissection, inadequate temperature control and irrigation in open air. The modified technique involves less surgery due to a new illumination system, controlled heating and a Mylar foil cover on the preparation to minimize room air influences. Temperature measurements and analyses of energy metabolism (ATP, CP, glucose, G6-P and lactate) are used as objective criteria of tissue normalcy. The microcirculation and metabolism are evaluated during anesthesia (α-chloralose) at rest as well as in hemorrhagic shock. In the resting state, muscle temperature drops to 28°C with the irrigation technique, whereas the Mylar technique keeps the temperature at 34–35°C. Neither technique causes deviations in normal metabolism. In shock, however, the temperature in the irrigated tenuissimus muscle falls 8–9°C below deep muscle temperature and there is a significantly attenuated metabolic response to ischemia, while the Mylar preparation follows the changes of unexposed muscle, both in temperature and metabolism.

Key words: Skeletal muscle microcirculation, vital microscopy, hemorrhagic shock, tissue environment, energy metabolism, Mylar

eral intravital microscopic techniques for study of the microcirculation in skeletal muscle have en described ([illegible] 1950, Zweifach & Metz 55, Hyman & Paldino 1962, Grant 1964, Baez & rlun 1967, Smaje et al. 1970, Baez 1973, Gray 73). These techniques are usually designed for all animals with thin muscles, easily accessible exposure and transillumination (Myrhage & udlická 1976). Most studies on general physiogical principles in central and peripheral vasilar control are, however, based on experiments n larger animals, usually dog or cat. Further ore, large animals have a rate, pattern and tolrance of metabolism that is more similar to human onditions than that of rodents and bats. In an t empt to combine the technical advantages of small animal preparations with the physiological advantages of the cat, Brånemark & Eriksson (1972) developed the m. tenuissimus model. Eriksson & Lisander (1972) also reported shock studies using this model. As we continued to use the m. tenuissimus preparation for further shock studies we came to realize, however, that the model in its original form was not representative for skeletal muscle in general, because the treatment of the preparation imposed serious alterations upon the local physiological environment as compared to the unexposed muscle.

Many reports (cf. Johnson 1973) emphasize the importance of careful tissue preservation in vital microscopic studies of the microcirculation. Ideally, the tissue under observation should have the

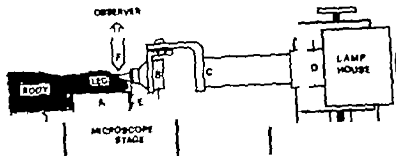


Fig. 2. Schematic drawing of horizontal illumination unit and animal preparation. *A*: leg elevation shelf; *B*: condenser with aperture diaphragm; *C*: extension tube; *D*: light-field diaphragm; *E*: prism holder fixed to shelf; *F*: objective in position above the tenuissimus muscle.

be stripping of the tenuissimus muscle were elimi-
1. After exposure of the external surface of the mus-
an 8–10 mm long incision was made parallel to, but
al millimeters away from the edge of the tenuissimus
in the fascia extending laterally in the plane of the
le. A pocket was then created in the loose areolar
e underneath the muscle for introduction of the tip of
the prism. In this way 5–6 mm portion of the tenuis-
muscle immediately distal to the popliteal fat pad
d be transilluminated without any distortion of the
cle.

During the preparation the muscle was kept moist by
Tyrode solution, pH 7.4, at room temperature in both
experimental groups.

Mounting and microscopy procedures. In both groups
animals were mounted resting on their right side on a
plexiglass plate with the left leg fixed to an elevated shelf
standardized manner to assure constant temperatures
of the tension in all experiments. Each experimental
group had its own specially designed plate, which was
attached to the cross-table of Leitz Biomed vital micro-
scope. In the *IG* the standard illumination system of the
microscope was used, while in the *MG* the light was intro-
duced horizontally into prism and reflected on its bev-
elled surface up through the tenuissimus muscle (Fig. 2
and 3).

The prism was made of plexiglass and cone-shaped with
a flat base and 45° bevel at the tip facing a flat horizontal
surface (3 × 8 mm) on which the muscle was positioned. A
thin mirror glass was cemented to the bevelled sur-
face. The dimensions of the prism were: base diameter 13
mm, tip diameter 6 mm and length 24 mm. The length was
chosen so that the focal plane of the condenser front lens
(UVL20, Leitz) would fall immediately above the hori-
zontal surface, thus the muscle preparation. The
unit was held by an adjustable, vertical plate and once in
position it was fixed to the shelf holding the leg. Thus the
prism became immobile in relation to the tenuissimus
muscle.

The light source was Leitz lamphouse (Model 100 Z)
equipped with light-field diaphragm. The condenser
(UVL 20, Leitz) was attached to the lamphouse by a
standard condenser holder and metal tube, 10 cm long.
This unit was mounted on separate stand placed beside
the microscope and positioned so that the surface of the
condenser front lens came in close approximation and
parallel to the base of the prism. The light source unit also
had to be coordinated with the movements of the micro-
scope cross-table. To observe the entire width of the mus-
cle belly the horizontal light beam had to be movable up
and down the bevelled surface. To allow this the light
source including the condenser was made vertically ad-
justable in relation to the prism. The light source unit was
also made adjustable horizontally in the same two direc-
tions as the microscope stage to allow focal adjustments
of the condenser light cone. With this system the illumina-
tion could be controlled according to Köhler's principle
for condenser transillumination.

For microscopy immersion objectives (Leitz) UO 11,
NA 0.25; UO 23, NA 0.55; UO×55, NA 0.80; UO 75,
NA 1.0 and UO 100, NA 1.00 were used.

During the experiments the microcirculation was docu-
mented from few areas of good resolution where all parts
of the microvasculature could be visualized. These areas
were recorded on video tape at pre-determined intervals
during the control period and before, during and after
bleeding.

Local tissue environment. In the *IG* expts. the observed
tenuissimus muscle was exposed to room air and kept
moist by intermittent irrigation with Tyrode solution, con-
taining 2% dextran 70, at room temperature. The local
temperature was recorded in the contralateral thigh mus-
culature and selectively on the surface of the exposed
tenuissimus muscle with a needle thermistor. In the *MG*
experiments the tenuissimus muscle was entirely covered
by a Mylar® foil soon after mounting (see Fig. 3). Mylar®
is transparent plastic film 12 μm thick, which is highly
impervious to gas diffusion (Severinghaus 1968), thus vir-
tually eliminating evaporation and environmental influ-
ence of room air PO_2 and PCO_2 on the exposed tissue. The
Mylar® adhered snugly to the moist surface of the prepara-
tion and caused no disturbances to the microscopic light
path.

On top of the Mylar® foil continuous drip of heated
Tyrode solution was administered by peristaltic pump.
The temperature of the internal tenuissimus muscle sur

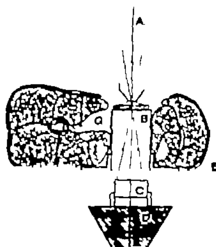


Fig 1 Cross section of cat hind leg with glass cylinder inserted for tenuissimus muscle transillumination according to the procedure used in the irrigation group *A* objective *B* glass cylinder with tenuissimus muscle on top *C* condenser front lens *D* condenser *E* elevated shelf for the leg *F* cross-sectioned thigh muscles *G* popliteal fat pad

same physical and chemical environment as before it was exposed This means that it should be atraumatically exposed and protected from external oxygen cooling and dilution or wash-out of local metabolic changes through external irrigation In an attempt to better conform to these preparation requirements Fronek & Zweifach (1975) described a modification of the tenuissimus muscle preparation A further improvement was recently reported (1977) by the same authors There is still a need however for direct metabolic data verifying the integrity of muscle tissue exposed for vital microscopy

The present paper details an extensive development of the tenuissimus muscle model for vital microscopy where tissue environment dissection and illumination procedures have been improved in order to obtain a better control of the preparation in terms of temperature physiological gas tensions interstitial fluid composition and resting state mechanics Cellular metabolic analyses of the exposed muscle and the unexposed contralateral muscle were used for critical evaluation of the physiological preservation To reveal and emphasize possible differences between the two sides the energy metabolism was also provoked using a standardized hemorrhagic shock procedure This condition also served to determine the usability of the model in shock experiments

The material is presented in the f[illegible] parison of quantitative metabolic data betw[illegible] original method as described by Bråne[illegible] Eriksson (197?) and the presented modifie[illegible]

MATERIAL AND METHODS

Animals and anesthesia Thirty-six young [illegible] weighing ? –5 kg were used in the study [illegible] induced by diethylether and maintained by [illegible] chloralose (approx. 50 mg/kg b w t.), suppleme[illegible] needed with small doses (0.25–0 5 mg) of i v [illegible] abolish twitching under the microscope Trache[illegible] was performed to ensure free airways but respira[illegible] not assisted A polyethylene catheter in the left [illegible] jugular vein served as inlet to the central venous [illegible] A similar catheter in the right common carotid arte[illegible] connected to a Statham transducer (P 23 AC) [illegible] turn was connected to a Grass polygraph for co[illegible] registration of systemic blood pressure Both c[illegible] were internally coated with heparin to prevent c[illegible] Body core temperature was kept at 37°C by a [illegible] connected to a rectal thermostat.

Dissection Muscle dissection procedures and sequent mounting for microscopy were different [illegible] two groups of experiments compared in this study

In the first group the irrigation group (IG), the te[illegible] nuissimus muscle was exposed and dissected according to [illegible] method described by Brånemark & Eriksson (197[illegible] minor modifications as indicated below This original [illegible] cedure included a holding suture in the lateral edge of [illegible] tenuissimus muscle and a 5–6 cm long freeing of the [illegible] nal surface of the muscle from underlying structures [illegible] siderable severing and retraction of thigh musculature [illegible] derneath the tenuissimus muscle as well as lateral [illegible] ing of the muscle itself was necessary to accommod[illegible] hollow plexiglass cone which covered the [illegible] denser system The transilluminated portion of the [illegible] was placed on top of this cone In our revision of [illegible] method the plexiglass cone was substituted by a [illegible] glass cylinder 10–1 mm in diameter and with a leng[illegible] match the thickness of the thigh This cylinder was p[illegible] on a plexiglass plate holding the leg and inserted [illegible] dissected tunnel through the medial thigh musculature [illegible] that the tenuissimus muscle came to rest on its top [illegible] (Fig 1) With this technique the holding suture and stretching caused by retraction were eliminated and [illegible] muscle specimen could be transilluminated using a st[illegible] dard Leitz (Series 600) condenser It should be [illegible] however that the internal surface of the tenuissimus [illegible] cle still had to be freed from its surrounding tis[illegible] distance of 3 cm to allow a loose positioning on the [illegible] cylinde

In the second group of experiments the M[illegible] (MG) the tenuissimus muscle dissection could be sig[illegible] cantly simplified s a direct consequence of the re[illegible] truction of the light conducting system (Fig 2 and 3) T[illegible] medial tunneling and retraction of surrounding musc[illegible]

Coating process by AB Amin [illegible] Bromma, Swede[illegible]

L. The phosphagen and glycolytic metaboli levels in the exposed and unexposed m tenuissimus resting conditions and in hemorrhagic shock

alues ± S E. are given in µmol/g protein Number of animals in parenthesis Irrigation and Mylar groups are d by IG and MG respectively P values computed by Wilcoxon test

		ATP	CrP	G-6-P	Glucose	Lactate
conditions						
preparation						
osed	(4)	38.9±3.7	184±14.2	1.50±0 33	35 4± 8 6	11.-± 3 1
xposed	(4)	36.7±3.0	185± 4.6	0.52±0 10	20 4± 3.0	9 1± 2.2
croscopy						
osed IG	(5)	34 2±4 7	177±24 9	1.26±0 60	39.5±10.3	13.5± 3.8
osed MG	(5)	42.1±7 0	202±33 0	1 46±0.96	47.3±10.4	18.8± 6.8
xposed	(10)	47 4±4.8	211±26.5	1.31±0 49	26 8± 4.0	11 6± 2.4
rhagic shock						
exposed IG	(6)	58.4±7.0	47±13 9	6.9±1.6*	35 9± 8.7*	31.3± 5.8*
exposed MG	(7)	54.5±6 5	198±19.3	8.9_1 7*	79 1±10.9	164.8±20.3
unexposed	(13)	66.7±5.6	205±20.2	18.7±3 0	68 5± 7 8	117 0± 9 4

) 05 vs unexposed muscle.
) 01 vs unexposed muscle.

ajor metabolic changes occurred in the blood of ol animals. In both groups of bled animals on ther hand there were marked changes in these al blood variables, indicating a significant shift rds anaerobic metabolism The mean elevation ood pyruvate and lactate levels was almost ical in the two groups of bled animals. On this ground it seems justified to assume that a simi- metabolic situation should be present in the ital musculature of the two groups as well.

e outcome of the metabolic analyses of tenuis- s muscle tissue is shown in Table 2. At the end preparative manipulation including mounting e tenuissimus muscle for microscopy there no significant differences in the levels of the ed metabolites between the prepared muscle the contralateral control muscle. After a 2 h rvation period in the microscope under resting tions, there were still no demonstrable differ in phosphagen or glycolytic metabolite levels between the exposed and unexposed muscle. The tissue metabolite levels were at this point similar to those registered at the end of the preparative procedures Furthermore no differences in the metabolic situation between the *IG* and *MG* could be deduced from these resting condition experiments.

When muscle perfusion was compromised through bleeding, however the levels of ATP and the glycolytic metabolites in the unexposed tenuissimus muscle were generally increased These findings which show no energy compound depletion but confirm the shift towards anaerobic metabolism obvious from the blood samples are in agreement with our previous findings concerning early metabolic changes in skeletal muscle caused by acute hemorrhage (Amundson & Haljamäe 1976)

The metabolic data from the unexposed tenuissimus muscles of the two groups of shocked animals showed no significant differences On the exposed side however the shock-induced metablic changes

le 3 Rectal and muscle temperature recording after 2 h of vital microscopy under resting onditions in hemorrhagic shock

ould be noted that the tenuissimus muscle temperature was externally regulated in the Mylar group (see Methods). s ± S D are given in °C

	Rectal temp.	Thigh muscles on unexposed side	Tenuissimus muscle on exposed side	
			Irrigation group	Mylar group
ting conditions	37	34 0±0.8	30 0±1 1	33–35
orrhagic shock	35 4±1.5	29 4±1 7	20 9±0.2	29–31

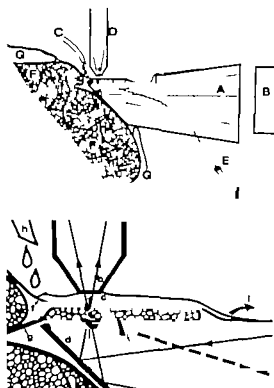


Fig 3 Upper drawing shows prism projecting light through the tenuissimus muscle according to the Mylar model *A* prism inserted under the tenuissimus muscle *B* condensor front lens *C* fluid drip *D* objective *E* thermistor connection *F* thigh muscles *G* skin flaps. Lower drawing shows cross section of the tenuissimus muscle on prism in enlargement *a* tenuissimus muscle *b* objective *c* warming immersion fluid *d* prism tip with light reflecting bevel *e* thermistor *f* Mylar cover *g* medial attachment fascia *h* drip spout *i* drainage of immersion fluid

face in the observation area was measured by a small thermistor attached to the tip of the prism The temperature of the deep musculature of the contralateral thigh was monitored by a needle thermistor The Tyrode drip was adjusted to keep the temperature in the observation area identical with that of the contralateral side It should be noted that the Tyrode fluid never came in direct contact with the exposed tissue

Experimental protocol To evaluate the cellular effects of the preparative procedure alone a *first set* of experiments was ended immediately after mounting by taking tenuissimus muscle biopsies for metabolic analyses from the exposed as well as the unexposed side Central venous blood was drawn at the same time for pyruvate and lactate determinations In a *second set* of expts. the same muscle and blood samples were taken after hours of intravital microscopic observations under resting conditions In a *third set* of expts a hypovolemic state was induced once the preparation had stabilized in the microscope for 20–30 min Hypovolemia was achieved by rapid withdrawal of 45% of the estimated blood volume (6% of b wt) through the arterial catheter The resultant shock state was studied for 2 h without any additional manipulation of blood volume The experiments were terminated after the [illegible] sampling procedures as described above.

Table 1 *Blood levels of pyruvate and lactate mmol/l ± S E in control animals and in the different shock groups*

Number of animals in parenthesis. Irrigation and [illegible] groups are indicated by IG and MG respectively

		Pyruvate		Lactate
Resting conditions				
End of preparation	(7)	0 074±0 018	(1[illegible])	[illegible]
2 h microscopy	(4)	0 063±0 011	([illegible])	[illegible]
Hemorrhagic shock				
2 h IG	(5)	0.2[illegible]5±0 034	(5)	[illegible]
2 h MG	(7)	0.268±0 04[illegible]	(7)	[illegible]

In the *first set* of expts only the irrigation model [illegible] used since results indicated good preservation [illegible] point In the second and third sets of expts [illegible] separate series with each model were performed [illegible] metabolic data from the exposed tenuissimus [illegible] each series were evaluated The data from [illegible] tenuissimus muscles within each experimental [illegible] combined regardless of preparation model [illegible] reference

Sample processing The muscle samples [illegible] w wt) were immediately immersed in liquid N_2 and [illegible] there until homogenization which was performed [illegible] μl of icecold 3 M perchloric acid After neutralization [illegible] 800 μl of 2 M $KHCO_3$, the samples were centrifuged [illegible] supernatants taken for fluorometric enzymatic [illegible] nation of glucose G6-P lactate CP and ATP levels [illegible] cording to previously described techniques (Haljamäe [illegible] Enger 1975) The pellets were used for protein [illegible] analysis of the samples, which served as a reference [illegible] dard for the metabolite levels expressed as μmol/[illegible] tein

In the blood samples pyruvate and lactate levels [illegible] determined using Sigma kits (726-UV and 826-UV)

RESULTS

In cats not subjected to hemorrhage a stable [illegible] ly unchanged blood pressure level was observed throughout the experimental period The hemorrhage resulted in an initial drop in the mean arterial blood pressure to 50–60 mmHg followed by a [illegible] pensatory regain to 80–90 mmHg after 30-60 [illegible] Towards the end of the 2 h period the blood [illegible] sure often exhibited a minor gradual decline [illegible] Amundson & Haljamäe 1976)

Blood levels of pyruvate and lactate of con[illegible] animals and animals subjected to hemorrhagic shock are shown in Ta[illegible] These data show [illegible]

...en exposed and unexposed muscle or be ... the two preparative models could be dem... ...ted. Despite this resemblance in metabo... ...he two models had very different micro... ...lar flow patterns. In the irrigation model ...was a brisk, apparently hyperemic flow while ...rocirculation in the Mylar group was charac... ...d by intermittency and generally slower flow ...ties. The hyperemia in the irrigation model ...ardly be explained by high metabolic demands ... due to the low temperature in this prepara... ...these should be reduced compared to the My... ...oup. We interpret the hyperemic reaction in ...rigation model as part of an inflammatory re... ...e elicited by substances released by the ex... ...ve preparative trauma involved in this model ...*ntrast*, the much reduced preparative trauma ... Mylar group results in an undisturbed mi... ...rculation which probably corresponds better ...e metabolic demands *of the tissue. A further* ...ort of good physiological preservation in the ... *group is the fact that* only with this model ...we been able to obtain vasoconstriction and ...tion of flow upon exposure of the muscle to ...ar oxygen tension (cf. *Arfors et al.* 1975, ...ng 1974, Prewitt & Johnson 1976).

...*ed situation.* In shock the hemodynamic ef... of vasoconstriction, capillary occlusion and ...lar stasis cause the balanced *nutritive tissue* ...sion to deteriorate and be substituted by a ... flow distribution with a large number of ...ted capillaries. These changes induce severe ...rbances in parenchymal cellular function. The ...ar metabolism is drastically altered due to ...xia and local metabolite accumulation causes ...*) fall*.

...*the irrigation* model it can be suspected that ...rrigation fluid diluted and cleared away the ...metabolites since only discrete changes could ...tected in the exposed muscle. On the other ..., it must be questioned to what extent such ... metabolic changes ever occurred in the ex... ...d muscle since the temperature drop kept the ...bolism on a low level, and external oxygen ...have reached the muscle fibers through the ...tion fluid and maintained an aerobic metab... ...n in spite of a drastically reduced blood flow ... microscopic observations under such con... ...ns become unreliable and would poorly re... the general status of muscle microcirculation. ...Preventing these externally mediated altera-

tions in exposed tissue composition and metabolism, the Mylar foild becomes increasingly important under shock conditions. Since it is impermeable to gases and adheres closely to the surface of the muscle only a very thin film of fluid excluded from contact with room air is formed outside the muscle. This minute volume can equilibrate with the tissue interstitium and a true *milieu interieur* representati e for the shock-induced changes is contained. Tissue temperature is controlled by the external drip which cannot affect tissue oxygen tension or interstitial composition.

In the Mylar model the metabolic alterations were similar in the unexposed and exposed tenuissimus muscle also in hemorrhagic shock. We take these results as strong evidence of a representative tissue environment in the exteriorized muscle. Consequently the Mylar preparation seems to safeguard the tissue from artifactual external influences and offer a reasonable physiological environment, which make microvascular flow analyses relevant and conclusive for microcirculatory events in skeletal muscle as a whole.

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differed considerably between the two preparation procedures When irrigation was used all glycolytic changes were less pronounced and there was a statistically significant difference between the ex posed and unexposed muscle The Mylar tech nique reduced these metabolic differences con siderably between the exposed and unexposed muscle A significant difference remained only in G6-P levels at the end of the 2 h shock period None of the exposure techniques affected the phosphagen content of the exposed tissue to any significant de gree however

The muscle exposure and shock procedures also resulted in temperature changes as shown in Table 3 Body core temperature decreased slightly in response to shock in spite of unchanged heating conditions As regards the muscle recordings it should be remembered that the temperature of the exposed muscle in the Mylar group was continually controlled by the Tyrode drip to match the muscle temperature of the opposite unexposed leg

DISCUSSION

Microscopy The quality of the vital microscopic picture obtained with the horizontal light conduct ing system is equivalent to that obtained with the original method for transillumination of the tenuis simus muscle (Brånemark & Eriksson 1972) The retained power of resolution is primarily achieved by maintaining a condenser system in the light unit which allows convergent light to pass through the muscle preparation A fiber optic illumination of the tenuissimus muscle which is described and pres ently used by several investigators (Fronek & Zweifach 1975 Tuma et al 1975) can be made more flexible delicate and convenient but its emitted parallel light does not permit the same degree of resolution

The illumination aperture of the present light source as determined by the condensor front lens is at the present 0 45 but it may be increased by the use of a shorter prism matching the Leitz 0 60/L11 condensor front lens An even higher numerical aperture might be obtained using a light pipe system with focusing lenses e g something of the nature described by Starr & Netto (1973) Condensing lens systems at the tip of a light pipe are however more space demanding than the tapered prism Such a system would therefore impose more tension and retraction in the tenuissimus muscle preparation It would also have to be moved in relation muscle when changing observation fields.

External environment It has recently be evident that exteriorized tissues prepared for microscopy can be seriously influenced microcirculatory flow characteristics by factors such as temperature oxygen tension irrigation procedures (Duling 1974 Chabr 1975 Prewitt & Johnson 1976). Since exp inevitable for high resolution microscopy of tissues the influence of such external factors be minimized to create circulatory conditions are representative for the tissue in its physiol surroundings Firstly the tissue specimen be allowed to cool off because metabolic well as vascular tone are highly temperature pendent (Grant et al 1931–33 Heisig 1968 Se ly oxygen availability must be controlled drastic increase in surface oxygen tension follows room air exposure of the preparation profoundly influence the flow rate of its super vessels the same vessels that of course most accessible for intravital microscopy Th the mode in which the tissue is kept moist importance in particular under the experi conditions where major changes in interstitial composition are known or anticipated to occur rrigation by artificial buffer salt solutions will ably dilute and attenuate such endog changes which in themselves play a role in vasoregulation Thus ideally the endogenous terstitium should be prevented from evap without external supplementation of fluid.

We feel that the Mylar foil presents an accep solution to control these external influences it efficiently excludes oxygenation drying in terstitial dilution in the preparation Still temperature control is fully transparent and no discernable tissue reaction during the vation period if applied gently and smoothly

The choice of quantitative criteria for tissue resentativity may of course be debated but opinion certain basic metabolic and physiol iables must be considered In addition to the ligatory temperature control we have therefore sen to study cellular energy metabolism since reactions are affected by variations in temper and oxygen availability as well as by local phenomena

Control situation Under resting condition significant differences in the analysed metab

'n exposed and unexposed muscle or be 'the two preparative models could be dem-ed. Despite this resemblance in metabo-he two models had very different micro-'ar flow patterns. In the irrigation model was a brisk, apparently hyperemic flow while crocirculation in the Mylar group was charac-l by intermittency and generally slower flow ties. The hyperemia in the irrigation model ardly be explained by high metabolic demands due to the low temperature in this prepara-hese should be reduced compared to the My oup We interpret the hyperemic reaction in rigation model as part of an inflammatory re e elicited by substances released by the ex e preparative trauma involved in this model. trast, the much reduced preparative trauma Mylar group results in an undisturbed mi-culation which probably corresponds better metabolic demands of the tissue A further rt of good physiological preservation in the group is the fact that only with this model we been able to obtain vasoconstriction and tion of flow upon exposure of the muscle to air oxygen tension (cf Arfors et al 1975 g 1974 Prewitt & Johnson 1976)

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In the Mylar model the metabolic alterations were similar in the unexposed and exposed tenuis simus muscle also in hemorrhagic shock. We take these results as strong evidence of a *representative* tissue environment in the exteriorized muscle Con-sequently the Mylar preparation seems to safe guard the tissue from artifactual external influ-ences and offer a reasonable physiological envi-ronment which make microvascular flow analyses relevant and conclusive for microcirculatory events in skeletal muscle as a whole

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relative analysis of microcirculatory and ular metabolic events in skeletal muscle ing hemorrhagic shock

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AMUNDSON B JENNISCHE, E. & HALJAMÄE, H Correlative analysis of microcirculatory and cellular metabolic events in skeletal muscle during hemorrhagic shock. Acta Physiol Scand 1980 108. 147–158 Received 11 April 1979 ISSN 0001-6772. Departments of Anatomy (Laboratory of Experimental Biology), Histology and Anesthesiology University of Göteborg, Sweden.

Skeletal muscle reactions to hemorrhagic shock were investigated in anesthetized cats (23) The tenuissimus muscle was exposed for vital microscopy and shock was induced by single-withdrawal of 45% of the blood volume. Muscle microcirculation, energy metabolism and cell membrane potentials were followed over 2 h period along with blood pressure hematocrit and blood leukocyte platelet, glucose pyruvate and lactate contents. Bleeding usually caused complete cessation of muscle blood flow for 5–20 min, while the animal compensated the blood pressure. Reflex constriction occurred in medium-sized but not in terminal arterioles. When flow reappeared marked maldistribution was evident in the capillary bed Flow remained in 30–50% of the capillaries, permanently or intermittently Leukocytes could be found lodged in many arrested capillaries and also adhering to venules in large numbers. Erythrocyte or platelet plugs were not seen in the muscle microvasculature Glucose and G6-P contents doubled and lactate increased 5-fold in muscle tissue during shock. CP was reduced by about 25% while the ATP-level remained unchanged Membrane potentials declined 12% in shock and the spread in potentials from adjacent fibers increased

Key ords Skeletal muscle microcirculation, vital microscopy hemorrhagic shock, energy metabolism, membrane potential high-energy phosphagens, lactic acid

blood flow reduction in peripheral tissues fol-ing a large acute hemorrhage, produces local urbances in tissue metabolism and function, se transport of oxygen and nutrients becomes fficient to meet the tissue demands. These ular changes rather than hemodynamic events constitute the pivotal factors in an ensuing ulatory shock condition (Zweifach & Fronek 5 Messmer & Sunder Plassmann 1975). To gain her understanding of the pathophysiology of ck at the cell level in peripheral tissues it thus ms pertinent to establish the precise characteris- of nutritive flow deficiencies and how fast and what xtent these affect tissue metabolism and ction

The largest tissue mass sustaining hypoperfusion in shock is the skeletal musculature The changes in total and nutritive blood flow occurring in striated muscle in shock have been extensively described in various experimental models (see e.g. Mellander & Lewis 1963 Grega et al. 1971 Appelgren & Lewis 1972, Eriksson & Lisander 1972b Hutchins et al 1973 Dawidson et al. 1979) Likewise disturbances in muscle metabolism and function during shock have been thoroughly investigated (see e.g. Le Page 1946a b Imai et al 1964 Lefer et al. 1969 Dmochowski et al. 1972 Chaudry et al. 1974) Primarily due to methodological difficulties little is known, however about the detailed correlation between microcirculatory phenomena and metabolic and functional derangements in skeletal muscle in shock. This report presents vital microscopic ob-

servations of muscle blood flow changes in hemorrhagic shock utilizing a modified m tenuissimus preparation (Amundson et al 1979) The microcirculatory data were combined with analyses of tissue metabolism and simultaneous recordings of muscle fiber membrane potentials (MPs) as an index of cellular function

MATERIALS AND METHODS

23 female cats, weighing 1 9–2 3 kg were used They were anesthetized by i.v α-chloralose (approx 50 mg/kg b wt) after ether induction Diazepam (0.2–0 4 mg i v) was given to about 50% of the animals to abolish muscular twitching Tracheostomy was performed but respiration was not assisted Blood pressure was measured through a heparin coated (Olsson et al 1977) PE-catheter in the right common carotid artery connected to a Statham 23 AC pressure transducer recording on a Grass polygraph An identical catheter was put into a central vein through the right external jugular vein for sampling and injections. Body temperature was kept at 37°C Anticoagulants were not given

Dissection and microscopy The left tenuissimus muscle was exposed for vital microscopy according to a technique described in a previous report (Amundson et al 1979) In short this technique makes high resolution observations of the microcirculation possible with reduced surgical dissection due to a horizontal illumination system with a prism transmitting convergent light through the muscle Furthermore an impermeable plastic cover (Mylar®) and a warming drip o er the exposed muscles are used to preserve and protect the internal tissue en ironment

Mixed light with a green filter was used with ×55 NA 0 84 and ×100 NA 1 00 immersion objectives for high resolution observations of cellular phenomena. Monochromatic illumination at 420 nm which increases the contrast of the red blood cells (RBCs) was used with ×11 NA 0 25 and ×23 NA 0 55 objectives for survey and flow pattern analysis The microcirculation was systematically observed throughout the muscle segment positioned on the prism (approx. 3×5 mm) and a few reference areas were selected for repetitive examination and documentation on ideo tape Recordings were made from these areas for a minimum period of ten minutes before during and at one and two hours after bleeding respectively

Flow analyses were concentrated on distributi e aspects of the nutritive perfusion An observation field visualizing 15–20 parallel capillaries was regularly used to document capillary flow patterns before and after shock Since pilot experiments had revealed widespread tand still of capillary blood flow in shock the number of non perfused capillaries i e vessels with zero RBC flow velocity was regarded to be the single most relevant variable to quantitate in the present investigation

Experimental protocol After a stabilization period of 35–60 min under controlled resting conditions in the microscope hypovolemia was induced through emular withdrawal of 45% of the calculated blood volume [illegible] b wt) The bleeding which was carried out by [illegible] syringe aspirations was made as rapid as [illegible] permitted without showing signs of cardia[illegible] and could usually be performed in 5–10 min The [illegible]lished hypovolemic state was studied for [illegible] further external alteration of the blood volume

Concomitantly with the video recordings of the circulation resting MPs were measured in [illegible]lar muscles and foreleg flexor muscles. The [illegible]ments were made with a modified Campion [illegible] (Campion et al 1969) described in a previous [illegible] jamie et al 1977)

Prior to microscopy a resting state reference [illegible] metabolite levels was taken from the biceps femoris muscle adjacent to the exposed tenuissimus muscle [illegible] end of the shock period the tenuissimus and biceps muscles were biopsied bilaterally in 7 expts. to [illegible] degree of agreement in reaction between exposed unexposed musculature In the remaining 16 [illegible] which were used for treatment studies [illegible] left biceps femoris muscle was biopsied through [illegible] incision at the end of the [illegible] h shock period

The biopsies (20–40 mg wet wt.) were immediately immersed in liquid N and kept there until homogen[illegible] which was performed at 0°C in 300 μl of 3 M [illegible] acid After neutralization with 800 μl of [illegible] M KHCO[illegible] samples were centrifuged and the supernatants [illegible] fluorometric enzymatic determinations of glucose-6-phosphate lactate CP and ATP levels [illegible]ing to previously described techniques (Haljamäe [illegible] 1975) The pellets were used for protein content [illegible] according to the method of Lowry et al. (1951) [illegible] determinations served as reference standards for metabolite levels expressed as μmol/g protein

At the instances of muscle sampling [illegible] venous blood was drawn for determination of [illegible] pyruvate and lactate concentrations. Sigma Kit[illegible] used for the analyses (14-UL 726-UV and [illegible] respectively) Prior to bleeding and after 1 and [illegible] shock blood samples (1–2 ml) were taken for hemat[illegible] arterial pH white blood cell (WBC) and platelet co[illegible]

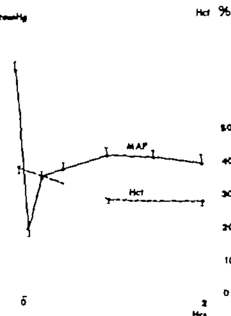
Significance tests were performed using the Stu[illegible] *t* test

RESULTS

Blood pressure pulse and temperature response

Fig 1 shows the computated averages of the [illegible] pressure graphs expressed in mean arterial [illegible]ure (MAP) Prebleeding MAP values were [illegible]ly in the 130–150 mmHg range and heart rate 180–220 beats/min The bleeding caused an immediate and profound pressure drop followed [illegible] prompt regain in pressure once the withdrawal [illegible] blood was completed In 30–45 min all animals [illegible] stabilized at a MAP of 60–90 mmHg This level

Coating proce[illegible] by AB A[illegible] [illegible]mma, S[illegible]



· 1 Changes in mean arterial pressure (MAP) and ·atocrit (Hct) during the 2 h shock period. The first ·a of the graphs represent prebleeding mean values ·-values are given

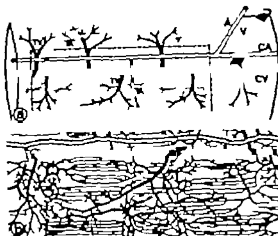


Fig 2 ( ) Principal vascular arrangement of the tenuissimus muscle Feeding arteriole (A) and enule (V) central arteriole (CA) and venule (CV) and transverse arterioles (TA) and venules (TV). (*b*) Magnification of the framed area in *a* showing semischematic drawing of the capillary bed. Only superficial vessels are included.

·tained over the h observation period with just ·ght decrease at the end in the typical case. Pulse already increased by the chloralose, rose only ·30 beats/min during bleeding and this level per ·d through the shock period. All animals sur ·d the initial bleeding of 45% of the blood vol· for 2 h in these experiments as opposed to a · fatality rate in a previous study when a 50% ·ding was used under identical conditions ·undson & Haljamae 1976) With regard to the ·rity of the shock condition it was noted, how· that irrespective of the level of blood pressure ·ulation no animal seemed to tolerate further ·d loss after the initial tap This was illustrated three animal which compensated the bleeding MAP levels above 100 mmHg. They became ·y table in blood pressure and died within a few ·utes when they were rebled 5–10 ml to deepen · hypotension after 30–50 min of hypovolemia. ·Animals that were kept alive after the 2 h obser ·ion period without any further interventions sur · ed for a varying length of time (range 0.5–4 h) · ore BP dropped in a manner similar to that of the ·bled animals.

In spite of unchanged heating conditions the rec· temperature dropped to 35°C after 2 h of shock, and the deep muscle temperature of the unexposed thigh went down from 34° to 29–30°C during the same period. The fluid drip on the exposed tenuissimus muscle was adjusted to produce a corresponding temperature.

M crovascular blood flow

The microvascular anatomy of the tenuissimus muscle as described by Eriksson & Myrhage (1972) will serve as basis for the description of flow pat terns (Fig. 2*a*) Nine categories of vessels will be discussed Feeding arterioles and venules (average resting diameter 110 μm and 140 μm, respectively) central arteriole and venule (70 μm, 90 μm) transverse arterioles and venules (20–30 μm 40–60 μm), 2 5 generations of terminal arterioles and collecting venules (8–15 μm 10–.5 μm) and the capillary network Capillary dimensions have a large spread 5.5 μm and 1 000 μm being the means of diameter and length, respectively All capillaries run parallel to the muscle fibers and most have 1–3 inter capillary anastomoses before reaching the venules (Fig. 2*b*) Dichotomous branchings and subsequent mergings within a single capillary are also regular features in this densely interconnected network Another noteworthy observation is the consistent

appearance of tributaries from two or more terminal arterioles to a specific capillary section Only rarely however do these tributaries stem from different transverse arterioles

The following results are based on the analyses of approximately 40 different observation fields in 23 tenuissimus muscles studied before and at 1 and 2 h after bleeding

Resting state The microcirculation in the well preserved tenuissimus muscle of the unbled resting animal was characterized by an on-and-off type of capillary perfusion with a very homogenous distribution The intermittency of flow was extremely sensitive however to mechanical disturbances (Amundson et al 1979) The slightest stretching or touching of the muscle would convert the on-off type of flow to a rapid continuous flow (cf Eriksson & Myrhage 1972) The complete on-off cycle was very variable in duration ranging from 4–40 s in different expts and the arrest period occupied ½–¾ of the cycle The flow-arrests which occurred simultaneously in all ramifications from a specific transverse arteriole clearly emanated from vasomotion in these vessels In contrast the more distal arterioles exhibited cessation of flow without any discernable constrictions Different transverse arterioles seemed to constrict and dilate independently of each other As has been reported before (Lindbom & Arfors 1978) those transverse arterioles which cross the muscle and continue to arborize in the adjacent connective tissues showed less flow variations and lacked arrest periods

During the flow phase the blood distributed to practically all capillaries with striking uniformity in flow velocity and during the no-flow phase all vessels remained open and capillaries and venules contained RBCs at all times

The RBC capillary transit time in homogeneously perfused undisturbed muscle segments averaged 3–5 s depending upon length and width of the individual capillary The RBCs passed the capillaries in single file with plasma gaps of 1–? cell diameters between them They did not form aggregates other than loose rouleaux which were mostly seen behind WBCs which usually passed the capillaries more slowly than the RBCs These rouleaux were immediately broken up when they reached the venules

WBCs were never a prominent feature in the resting state capillary circulation They were most frequently found when specifically looked for in the most distal capillaries of a given network, [illegible] that they were carried by the axial stream [illegible] arterioles The WBCs passed the capillaries [illegible] 0–50 % lower velocity than the RBCs. WBCs [illegible] not seen to block single capillaries other than [illegible]sionally in the normal state whereas a [illegible] adherence to the venules always occurred [illegible] time

Hypovolemic state Hypovolemia caused [illegible] changes in the microvascular flow pattern [illegible] tenuissimus muscle During bleeding, [illegible] verse arteriole and its distal branches were [illegible] for observations of the immediate response [illegible] ½–⅓ of the total bleeding volume had been [illegible] first wave of vasoconstriction occurred, [illegible] flow cessation Typically this response was [illegible] 15–30 s duration followed by a rapid onset of [illegible] As bleeding continued such an interchange [illegible] flow and no flow was evident for a few minutes [illegible] a complete circulatory arrest occurred in the [illegible] muscle including the central arteriole The [illegible] period usually lasted 5–?0 min A covariation [illegible] be demonstrated betweeen the tenuissimus [illegible] blood flow and the systemic blood pressure [illegible] after bleeding Thus those animals which [illegible] relatively longer cessation of muscle blood [illegible] managed to compensate in blood pressure [illegible] readily and to a higher level than those [illegible] had no or merely a short period of muscle [illegible] flow cessation The vasoconstriction mediating [illegible] flow inhibition during and after bleeding [illegible] in the central and even more pronounced [illegible] transverse arterioles while the more distal [illegible]lar branches did not constrict During this [illegible] period the transverse arterioles appeared as [illegible] lines without any observable lumen while [illegible] constricted vessels looked like pale bands [illegible]ing only plasma At the onset of recirculation [illegible]al short constrictions were seen along the [illegible]verse arterioles as the solid constriction [illegible]ly gave way to flow in a wavering manner [illegible] became continuous

Once the flow had restarted after bleeding [illegible] characterized by considerable inter and intra[illegible]vidual variations Distributional defects in [illegible]sion i e patchiness of flow occurred on [illegible] levels The synchronous on-and-off pattern of [illegible] resting state had always vanished in this [illegible]volemic shock state

In the proximal pre-capillary vessels flow [illegible] generally continuous but [illegible] erved trans[illegible]



... Photographs of muscle capillary circulation, taken ... the TV-screen. The maldistributed capillary perfu- ... shock is evident from this area, photographed be- ... (A) and 1 h after bleeding (B). Arrows indicate ... ctions of flow in perfused capillaries

...rioles remained narrower during the shock ...od than in the unbled situation. In few trans- ...e arterioles the early short constrictions and ... motion persisted and flow was not uniform ...gh rarely stopped. Again in other transverse ...rioles standstill of flow prevailed throughout the ...rvation period, evidently as a consequence of ...ound constriction. This latter phenomenon, ...h left large areas of tissue unperfused, consti- ...d the first level of patchiness between flow and ...low. It was prominent in some animal but ...gnificant or absent in most.

...h the distal arterioles small flow variations could ...ys be detected, plasma gaps increased in ...mber and length and the blood failed to enter all ...minal branches seen perfused in the resting state. ...rt rouleaux formations became more frequent ... diameters and flow rates were not significantly ...anged in relation to pre-bleeding values in the ...ssels at this level. The number of unperfused ...minal branches had no relation to the distance ...m the feeding transverse arterioles i.e. proximal sidebranches had a similar number of arrested terminal arterioles as distal sidebranches. In no instance was a cell or a cellular aggregate seen to block a terminal arteriole. The unperfused terminal arterioles created the second level of patchiness of flow and this was present in all transverse arteriolar networks studied.

At the capillary level the overall number of perfused vessels was usually reduced to 30–50% of all visible capillaries at any one time during the 2 h shock period (Fig. 3). In comparison the unbled resting state constantly exhibited a perfusion of about 90% of the capillaries. This capillary maldistribution which constituted the third level of patchiness in the muscle microcirculation during shock, was characterized by the absence of blood flow in a fraction of the capillaries from a certain terminal arteriole in itself continuously perfused. In such a capillary network the following observations were made:

1. One specific group of capillaries was constantly perfused. The linear flow rate in these exhibited a range of variation much larger than that seen in the resting state.

2. A second group of capillaries shifted between flow and no flow at irregular intervals varying from seconds to several minutes in duration.

3. A third group of capillaries were invariably arrested when observed.

4. WBCs generally appeared in greatly increased numbers in the capillary bed, often impeding flow for seconds to minutes as they passed individual capillaries. During these retarded passages each WBC was always followed by a dense rouleaux of red cells giving the cellular column the appearance of a train as it moved.

5. One or several WBCs were seen in a number of the permanently arrested capillaries. These WBCs, which were difficult to discover due to their very low contrast, were usually seen wedged against bulging endothelial cell nuclei along the course of a capillary or jammed in a capillary opening at a branching point.

6. Capillary perfusion changes concurrent with blood pressure variations occurred. Thus at the peak of blood pressure compensation one hour after bleeding a significantly larger percentage of capillaries was perfused than after two hours when blood pressure had started to decline.

7. Platelet or erythrocyte-platelet aggregates were never seen in the capillary network. Thus

capillary plugging by these blood constituents alone could not be demonstrated

At the postcapillary level blood flow was substantially altered over the course of the observation period During the initial total ischemia constriction in the central and transverse venules was prominent but it subsided as soon as a continuous flow reappeared in the arterioles Hereby flow became continuous in the venular sections as well and during the remainder of the hypovolemic period diameter changes were not evident in any venules in comparison with prebleeding values Instead a major feature in venules was a progressive adherence of WBCs which totally paved the venular walls in the latter part of the observation period This uneven luminal reduction made the blood flow in a serpentine fashion between the WBCs which effectively broke up rouleaux (Fig 4) The flow velocity was often very low but RBCs did not aggregate or attach to venular walls Hence primary venular stoppage of flow due to cellular congestion was an uncommon finding

Emigration of WBCs from collecting venules was frequent in late shock Extravasation of RBCs however was rare and platelets were only observed among adhering venular WBCs Collapse was never observed in any venular segment

Membrane potentials

MP changes during the hypovolemic period are shown in Fig 5 There was a significant decline from a resting state value of −90 mV to about −80



Fig 4 Photograph of transverse arteriolar (A) and venular (V) segments in the tenuissimus muscle in shock taken from the TV screen The ma si e adherence of WBCs (W) to the venular walls and the undulating central blood flow in the venule are typical features in shock.

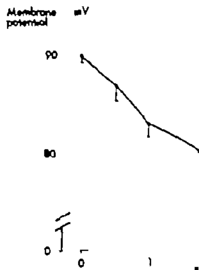


Fig 5 Drop in muscle fiber membrane potential the 2 h shock period The starting point of the represents the prebleeding mean value S E are given.

mV after 2 h in the typical case The decline linear but appeared to be more pronounced the first hour of shock The S E values as the shock state progressed as an expression larger spread among measurements in the fused muscles The increased spread coupled to the blood pressure reaction shock Thus variation in MP among adjacent cle fibers appeared more pronounced in the mals that suffered the deepest blood pressure reduction

Muscle metabolism

The results of the metabolite analyses are Tables 1 and 2 In Table 1 the tenuissimus reactions to shock are compared with changes the adjacent biceps femoris muscle Values the unexposed biceps femoris muscle before are included as reference Moreover the effect exposure can be evaluated on the basis of samples Indeed these effects were slight significant disparity only in the exposed simus G6-P content The other analyses that the metabolic reactions to shock were similar in the two muscles Once this similarity established sampling was limited to the left femoris muscle after shock These results are sented in Table ? as the mean total and percent difference in pre to posthemorrhage values pattern of change was identical in both (Tables 1 and) The was unchanged

| *Tissue metabolites i left biceps f moris muscl before bleeding and in right and left tenuissimus eps femoris muscles after 2 h of shock*
re for microscopy was on left side where shock samples were taken from portions of the muscles covered by the ol Means from 7 expts ± S.E. values are given in μmol/g protein

Control samples	Shock samples			
Biceps femoris	Tenuissimus		Biceps femoris	
Unexposed	Exposed	Unexposed	Exposed	Unexposed
55.2±5.8	54.5±5 5	58 6± 6 8	58 8± 6.1	60.1± 6.3
44 ±8 2	198 ±19.3	199 ±32.7	186 ±29 6	200 ±25 7
7 5±0.7	8.9± 1 7	15.3± 1.9	17 1±1.9	21.3± 3 4
31 0±6 0	79 1±10.9	69.3± 9 0	64.9±12.2	53 7±10.1
21 7±2 1	165 ±20.3	124 ±11.8	130 ±16.4	111 ±14 4

the CP pool was reduced by about one quar glycolytic metabolites all demonstrated sig- nt increases, which in the case of lactate was

2 *Mean metabolite changes in biceps femo- scle after 2 h of hemorrhagic shock*

es are expressed in concentration difference and in t of prebleeding values Changes in blood (B) glu- pyruvate and lactate are expressed in the same unco- E -values given Significance level ** $P<0.001$ vs fference. ns not significant

		Per cent	N of ani- mals
	μmol/g protein		
	45±2 74**	+5 5± 4 95	15
	−49 ±6 2***	25.2± 3 63	15
	8 7 ±1 43 **	185 ±36	15
	22.9 ±2.9***	+130 ±23	15
	69 8 ±10 8***	665 ±108	15
	m/mol/l		
cose	12 3 ±2 1***	+111±22	9
ruvate	0 155±0 024**	+314±60	16
ate	4 23 ±0 81***	418±94	17

Blood analyses

Changes in central venous blood concentration of glucose pyruvate and lactate as a consequence of shock are included in Table 2. Glucose increased twofold and pyruvate and lactate content went up three and four times respectively Thus the lac tate–pyruvate ratio increased from 16.7 to 24.5 during the observation period indicating excess lactate formation.

The hematocrit changes are included in Fig. 1 Hemodilution was significant during the blood pres- sure compensation phase suggesting transvascular absorption of fluid. During the second hour of shock the hematocrit remained stable Arterial pH varied only insignificantly within the 7.30–7 40 in- terval over the shock period Concomitant determi- nations of pO_2 and pCO_2 indicated respiratory com- pensation

WBC and platelet counts are presented in Table 3 The number of WBCs increased significantly af ter 2 h of shock, whereas changes during the first hour were insignificant due to very large interin- dividual differences As regards platelets, the varia- tion in initial numbers as well as in subsequent

e 3 *Mean blood leukocyt nd platelet counts before and after 1 and 2 h of shock*

two righthand columns show mean percentage changes of prebleeding values in the shock samples. Number of als in parenthesis S.E.-values given. Significance levels. $P<0.05$ $P<0.001$ vs. zero difference. ns=not ificant

	Before shock	1 h	2 h	%Δ 1 h	%Δ 2 h
ocytes	9 490 ±1 765 (15)	7 940 ±1 570 (8)	17 720 ±3 150 (15)	+44 6ns ±34 (8)	+121 ±25 (15)
	358 000 ±53 300 (11)	279 000 ±55 700 (8)	264 000 ±34 000 (11)	−14.5ns ±11.0 (8)	−20.2* ±8 4 (11)

reaction was considerable but definite consumption during shock was not seen

DISCUSSION

Experimental model

The morphological resemblance between the tenuissimus muscle and thicker hind leg skeletal muscles in the cat has been documented by Myrhage (1977) The vascular arrangement of the tenuissimus muscle (Fig 2) is found as a "basic vascular unit" in 3-dimensional form in larger muscles and its muscle fiber composition and capillary/fiber ratio are quite similar to those of other phasic muscles such as the biceps femoris and the lateral gastrocnemius muscles

The tenuissimus muscle preparation used in this study was modified in detail to minimize the artifacts of trauma and exposure Its vasculature has been shown to respond to changes on oxygen tension and reaction highly sensitive to mechanical trauma (cf Lindbom et al 1976) Furthermore the exposed muscle reacts metabolically to hypotension in a manner closely similar to that of the unexposed contralateral muscle (Amundson et al 1979)

Using the same muscle preparation with a Saran wrap cover Fronek & Zweifach (1977) found that one sided exposure and microscopy for 2 h caused no change in blood flow ratio between the left and the right tenuissimus muscle when compared with the bilaterally undissected control situation

The slow homogeneous on-off capillary flow pattern seen in the resting state also seems to indicate an undisturbed tissue preservation The observed mean capillary transit times (3–5 s) agree with direct measurements in the cat sartorius muscle (Burton & Johnson 197?) as well as recent morphological (Honig et al 1977) and indicator clearance (Paaske 1977) computations The intermittent flow characteristics have also been recently demonstrated in muscle microcirculation (Prewitt & Johnson 1976 Tuma et al 1974) Control studies on tenuissimus muscle energy metabolism (Amundson et al 1979) have clearly shown that this type of flow does not leave the tissue insufficiently perfused In all probability the intermittent flow represents a normal and healthy mechanism by which capillary recruitment and volume flow are limited at rest

On the basis of all these findings it seems justified to assume that the present model is repre[...] for skeletal muscle tissue and vasculature [...]eral

Shock model

When deciding on experimental shock pa[...] resemblance to clinical hemorrhage w[...] to cardiovascular and hormonal reactions [...] guideline On the basis of these considera[...] single withdrawal bleeding defined by v[...] shed blood was chosen as it allows the expe[...] benefit from a compensatory recovery [...] pressure and blood volume after comple[...] hemorrhage By this method the hypovole[...] duces a sequence of reactions in the cen[...] peripheral circulation which is innate to the [...]ganism and consequently similar to [...] clinical hemorrhage (cf Zweifach & Fronek [...]

Blood pressure reaction

The autonomous recovery in blood pres[...] evident from Fig 1 is a clearly diphasic [...] The fast increase follwoing the posthe[...] nimum is mainly the effect of reflex vaso[...]tion and redistribution of blood volume (cf [...] 1967) In this phase complete flow cessation [...]ally prevailed in the tenuissimus muscle The [...]sequent slow increase in MAP over the first [...] shock probably results from plasma volume [...]sion through transcapillary absorption Early [...] phase flow permanently reappeared in the te[...]mus muscle According to Järhult s data (19[...] cumulative fluid absorption from muscle [...] to ? ml/100 g in the first hour of hemorrhage [...] Considering the likeness in bleeding sever[...] hyperglucemia response it is reasonable to [...] a similar magnitude of absorption in our e[...]ments A hemodilution of that order would [...] account for the decrease in hematocrit obser[...] the first hour of shock This finding challeng[...] conclusion of Bäckström et al (1971) who po[...] sequestration of RBC aggregates in the sk[...] muscle vasculature in addition to hemodilu[...] account for the drop in hematocrit after ble[...] However no RBC aggregates of any sort othe[...] rouleaux could be observed in the muscle mi[...]culation following hemorrhage in the present s[...]

Microcirculati m in shock

A profound reduction in the total blood f[...] skeletal muscle in respons[...] acute hemor[...]

en consistently demonstrated and accurately
od in whole organ preparations (see e g
1973). However to elucidate the cause of
metabolic disturbances in hemorrhage it is
essential to determine the capillary distribu-
flow The permanent flow cessation in single
es has the most serious impact on the tissue
respect. Since this phenomenon was striking-
ouent in the present experiments it was
ly recorded and quantified Evidently the
effects of the nonperfused capillaries could
compensated by the flow in the capillaries
nained perfused after bleeding (Tables 1 and
art from complete stoppage of flow the
ynamic characteristics of the microvessels
re appear less relevant to quantitate in rela-
muscle metabolism in shock. An emphasis
atistical presentation of hemodynamic var
e g diameter and flow velocity of the per
vessels may even convey a spurious impres-
f uniformity inconsistent with the extreme
geneity of the microcirculation in shock.

n evaluating the observation of complete
all of muscle blood flow during and im-
ely after bleeding the comparatively small
ter of the feeding arteriole of the tenuissimus
e (approx. 100 μm) and the size of the obser
area (approx. 3×5 mm) must be kept in mind
organ studies of muscle blood flow during
ng do not report a complete cessation of flow
nder & Lewis 1963 Gregg et al. 1971 Järhult
Kovách 1973). Conversely these studies in-
that some flow always remains Provided
he experimental conditions of the different
rations are comparable it seems reasonable
ver to reconcile the whole organ data with the
nt results on the basis of regional differences
sovconstriction The theory would be that a
rity of the resistance vessels, the size of the
ng and transverse arterioles of the tenuissimus
le become entirely constricted in response to
e bleeding while a few arbitrarily dispersed
ls remain perfused In a few experiments one
ese perfused vessels happened to be included
e observation area, which in these cases con-
ently failed to exhibit a complete stoppage of
. The reason for this incomplete coverage of
pathetic vasoconstriction during bleeding is not
to the authors.

he observed pattern of arteriolar constriction
ng and immediately after bleeding, which leaves
the terminal arterioles virtually unconstricted,
agrees with the detailed study by Hutchins et al
(1973) in the rat cremaster muscle, and with obser
vations in the tenuissimus muscle under sympathet
ic stimulation (Eriksson & Lisander 1972*a*) This
difference in arteriolar reaction may be due to varia-
tions in density of sympathetic innervation along
the arteriolar tree or to a competitive beta adrener
gic dilator response in the terminal arterioles
(Lundvall & Järhult 1976). The marked dilatation of
the transverse arterioles in the tenuissimus muscle
soon after bleeding, reported by Eriksson & Lisan-
der (1972*b*) could not be reproduced in our experi-
ments

When blood flow reappeared in the muscle after
the bleeding, maldistribution was evident from the
start due to the reduction in perfusion pressure and
greatly varying degrees of sustained vasoconstric
tion In addition a very striking finding and prob-
able cause for maldistribution was the capillary en-
trapment of WBCs seen in increasing numbers over
the first hour of hypotension Since this phenome
non may be of quantitative as well as qualitative
importance in shock, the question of WBC plugging
of capillaries was further explored in a separate
study (Bagge Amundson & Lauritzen 1979)

The unchanged WBC count after 1 h of shock
(Table 3) indicates that general sequestration and
mobilization of WBCs balanced over this period.
After h of shock mobilization dominated how-
ever concomitant with the abundant appearance of
adhering WBCs in the venules. We interpret the
WBC adherence and emigration as an inflammatory
reaction possibly elicited by proteases released
from the hypoxic muscle in shock, activating the
complement system (cf Hill & Ward 1969) Com-
plement has been shown to increase WBC adhe
siveness (Fehr & Jacob 1977 Hoover et al 1978)
and to be chemotactic for WBCs (Ward & Newman
1969)

Due to the inherent venular capacitance and the
reduced volume flow rate through arteriolar con-
striction in the muscle in shock the WBC venular
reaction did not seem to appreciably impede blood
flow however since transient increases in flow
were easily conducted through the venules without
signs of stasis

Several investigators have concluded a maldis-
tributed muscle microcirculation in hemorrhagic
shock, using indirect methods (Bäckström et al.
1970, Appelgren 1972, Dahlgren 1979) as well as

vital microscopy (Eriksson & Lisander 197?b) In the Bäckström and the Eriksson reports erythrocyte aggregation and microthrombi formation are proposed to cause the capillary occlusion In the present study neither RBC aggregates nor microthrombi were seen possibly due to the fact that the tissue trauma was minimized in comparison with the more elaborate preparations used by other investigators (cf Swank et al 1964 Heideman et al 1979)

Regardless of cause however it does not seem warranted to regard the heterogeneity of capillary flow as an expression of functional shunting (cf Eriksson & Lisander 197?b Zweifach & Fronek 1975) in the sense that blood in perfused capillaries traverses the muscle without optimal transendothelial exchange Such a concept would require a highly increased linear flow rate in the patent capillaries which was not seen Thus there seems to be no reason to suspect that the capillaries that do have flow in shock are shunting the blood without letting it perform its nutritional task in a normal way Due to the large number of unperfused capillaries however multiple sections of the muscle tissue are still inadequately nourished as indicated by the increase in anaerobic metabolism and the depressed MPs

Although there was often a moderate decline in MAP towards the end of the shock period no conclusive changes (i e dilatations) were observed in the microvascular tone This was true also for those animals that suddenly decompensated and died within the observation period indicating absence of arteriolar dilatation in muscle even in terminal shock (cf Grega et al 1971)

Indices of tissue function in shock

It is evident from the metabolic analyses that the flow disturbances observed in the microscope were severe enough to interfere with aerobic metabolism in the tenuissimus muscle As shown in Table 1 the adjacent biceps muscle and the unexposed muscles of the other leg responded to shock in a closely similar manner Most significantly there was a pronounced augmentation of glycogenolytic pathways in the shock state This resulted in a marked lactate accumulation and a gradual increase in blood lactate pyruvate ratio While this shift towards anaerobic metabolism took place in the tissue the microcirculation was characterized by persistent arteriolar constriction and flow cessation in 50% or more of its capillaries. It may be calc... comparison with Sahlin s data (Sahlin et al ... the relationship between total muscle pH ... tate + pyruvate content that the ... levels in our experiments would correspond ... intracellular pH of 6.6–6.8 after ? h of shock

The decrease in CP content also indicates ... turbance in energy metabolism. The ... CP to ATP may be caused by hypoxia as ... a shift towards breakdown in the creatine ... equilibrium due to the low pH in the ... et al 1975) The extent of anaerobiosis ... seem pronounced enough however to ... ATP level in the resting muscle during the ... period This finding is supported by earlier studies (Chaudry et al 1976, Amundson & Haljamäe 1976)

The decrease in MPs points to a disturbance of the normal control of fluxes and charges of the cell membrane This is in agreement with ... ous studies showing shock induced disturbances of the electrolyte transport characteristics of muscle cells resulting in a loss of intracellular potassium and an increase in interstitial ... (Hagberg et al 1968 Haljamäe 1970). The electrolyte transport disturbance seems to be related to the extent of tissue lactoacidosis rather than to changes in tissue phosphagen content Thus the membrane charge a complex energy-dependent phenomenon degenerates in spite of an ... ATP content in the tissue The exact cause of ... derangement is yet unknown although the correlation to tissue pH and lactate content ... to an active role of these factors in the ... (cf Jennische et al 1978)

The more pronounced decline in MPs during the first hour of shock coincides with the increase in number of capillaries without flow The ... heterogeneity in nutritive blood flow could ... for the increase in scatter among MPs from ... fibers seen in the hypovolemic period This ... sion is supported by earlier findings in ... cle during splanchnic shock (Haljamäe et al ... The theory is that fibers farthe t from the ... vessels suffer most from hypoxia and conseq... decline more in potential

In c nclu i n

The skeletal musculature is subjected to pr... changes in blood flow metabolism and f... during acute hemorrha ... In additio...

...duction in volume flow a marked capillary ...bution appears. In vivo observations indi... WBC plugging is involved in this distur... the nutritive blood flow Platelet or RBC aggregates are not seen in the vasculature ... adherence becomes prominent in all ven... ...st aerobic metabolism is perturbed, as evi... ...y tissue lactate accumulation and degrada... ...P content. Muscle fibers fail to uphold their ...ne potential Precapillary vascular tone is ...ed over the observation period although ...al animals fail to survive the shock period

...ical assistance of Mrs Carin Almiger is grate... acknowledged

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blood cell deformability and plugging
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BAGGE U AMUNDSON B & LAURITZEN C. White blood cell deformability and plugging of skeletal muscle capillaries in hemorrhagic shock. Acta Physiol Scand 1980, 108, 159–163 Received 11 April 1979 ISSN 0001-6772. Laboratory of Experimental Biology Department of Anatomy University of Göteborg, Sweden.

The cause of maldistributed capillary perfusion in hemorrhagic shock was investigated in a combined in vivo and in vitro study. The cat tenuissimus muscle was observed by vital microscopy before and after withdrawal of 45% of the blood volume. The induced shock conditions reduced the number of perfused capillaries about 50%. Close examination of the scattered, nonperfused capillaries showed that they remained open and almost invariably contained one or several leukocytes. These cells were usually located at the bulgings of endothelial cell nuclei, and when occasionally dislodged, recirculation immediately oc curred. Platelet or erythrocyte aggregates were not seen in the microvasculature in shock. T test whether the trapping of leukocytes reflected an impaired deformability of these cells in shock or was merely pressure dependent phenomenon, deformability studies were performed on leukocytes in vitro. Central venous blood was drawn before and 2 h after bleeding and leukocytes were separated by sedimentation. The passage-time of single leukocytes through a glass capillary stenosis at constant driving pressures was used as an index of cell deformability. No qualitative changes in stiffness were found after shock. The distribution of cell passage-times indicated however that the stiffest portion of the leuko-cyte population was removed from the circulation in shock.

Key ords Leukocyte deformability leukocyte plugging, hemorrhagic shock skeletal muscle, vital microscopy

rrhagic shock leads to a reduced and maldis-d blood flow in skeletal muscle (Appelgren & 1972, Dahlberg 1979). Vital microscopic ob-ions reveal that a large number of the capil-in the skeletal muscle cease to be perfused son & Lisander 1972). It has been suggested the microvasculature becomes occluded by et and ed blood cell (RBC) aggregates Åström et al. 1971). However recent tudies n laboratory with an improved technique for microscopic studies of the tenuissimus muscle show widespread cessation of capillary flow absence of microthrombi (Amundson et al. b). Instead observations were made which in-e that stoppage of the capillary flow might be ed to white blood cell (WBC) plugging. These observations prompted the present investigation which was aimed at a more detailed analysis of the behaviour of the WBCs in the capillary bed in hemorrhagic shock. In addition to the in vivo observations a preliminary in vitro study was included to analyse whether the deformability of WBCs changes in shock.

MATERIALS AND METHODS

11 female cats, weighing 1.9–2.3 kg were used—5 for in vivo observations and 6 as blood donors for in vitro expts. All animals were anesthetized with α-chloralose i. (50 mg/kg b wt) after ether introduction. Tracheotomy was performed but the animals breathed spontaneously. Body temperature was kept at 37°. In all animals the right common carotid artery was cannulated for blood pressure



Fig 1 End of glass capillary with stenosis—magnified below (inner diameter 5 μm)

measurements and a central venous catheter was introduced through the right external jugular vein for injections and blood samples Shock was induced by withdrawal of 45% of the estimated blood volume (6% of b wt) over a period of 5–10 min (cf Amundson et al 1979*b*)

In vivo experiments

The left tenuissimus muscle was exposed for vital microscopy according to a previously described technique (Amundson et al 1979*a*) After exposure a light conducting prism was put under the muscle and a plastic cover (Mylar®) was placed on the muscle to exclude external environmental influences

Observations of the microcirculation were made in a Leitz Biomed microscope with water immersion objectives ×23 NA 0.55 ×55 NA 0.84 and ×100 NA 1 00

Before bleeding representative microvascular segments in the tenuissimus muscle were selected and the microcirculation in the areas was recorded on video tape Further since it was known from previous expts that many capillaries may be very difficult to discover in the vital microscope in shock due to maldistribution of the blood flow drawings were made of the capillary network to facilitate identification of all vessels in the shock phase

After bleeding the selected areas were reexamined at intervals for 2 h and the number of perfused capillaries and the directions of flow were compared with the pre bleeding recordings Detailed observations were made on all non-perfused capillaries

In vitro experiments

An index of WBC deformability was obtained by measuring the time required for individual WBCs to deform and pass through a narrow stenosis in a glass capillary at a constant driving pressure

Glass capillaries with a stenosis were produced according to a method described by Bagge et al (1977*a*) (Fig. 1) The diameter at the narrowest point of the stenosis was kept between 4 0 and 5.5 μm The capillaries were glued to glass slides and two basins were made of Epoxyce-ment—one around the stenosis to contain immersion fluid for the objective and one around the distal end of the capillary for the WBC suspension. The glass slide with the capillary was placed on the stage of a Leitz vital micro-scope which was connected to a TV-camera, a video recorder and a video timer (accuracy 1/100 s) ... tions and video recordings of the WBC passage ... the stenosis were made with a water immersion ... ×55 NA 0 84 at room temperature (20-22°C)

The glass capillary was connected by PE ... reservoir which could be adjusted vertically ... graded scale for application of different hydrostatic pressures The whole system was filled with Tyrode pH 7 4

WBCs were obtained from blood drawn from the catheter before and after 2 h of shock. Five ml ... was anticoagulated with 0 95 ml 15% K_2-EDTA ... WBCs were separated from the RBCs by sedimentation in ESR tubes for one hour at room temperature

A few drops of the WBC suspension were put ... of the capillary and WBCs were sucked into the ... at a constant negative pressure The pressure ... selected to obtain deformation times of the ... nitude as observed normally in vivo (0 5–3 s, cf ... Brånemark 1977) and ranged from 10 mm ... mm H_2O Both samples in each expt were tested ... same capillary using the same suction pressure

The deformation time (DT) for consecutive WBC ... ing through the stenosis was measured by ... analysis of the video tapes. DT was defined to ... moment a WBC hit the walls of the tapered ... stenosis and to end when the whole cell had p... midpoint of the stenosis (Fig 2) To determine ... sible occurrence of plasma viscosity changes ... would influence the deformation data, particle ... velocities were measured for each sample at ... in a defined segment of the capillary

Mean deformation time (MDT)±S E. was calc... all samples. In each experiment the MDT for WBC ... tained before shock was compared with the ... WBCs collected after 2 h of shock To allow com... between different experiments differences in MDT ... expressed as percentage of the MDT obtained ... shock

RESULTS

In vivo observations

The rapid bleeding led to a profound drop in arterial blood pressure (MAP) from about ... mmHg to 30–50 mmHg When the bleeding ... completed reflex compensatory mechanisms ...



Fig 2 The WBC def... the time required for ... and *b*

[illegible] WBC mean deformation time (MDT)±S.E. before (N) and after (S) 2 h of shock in the six in vitro

[illegible] were drawn from central venous blood. Percentage change in MDT during shock in the right hand column. [illegible] stenosis diameter (Ø), deformation pressure (P) and MDP are expressed in µm, mm H_2O and seconds, [illegible]ely. The number of cells analyzed in each sample is given in parenthesis

Ø	P	MDT (N)	MDT (S)	%Δ
5.9	−30	1.95±0.14 (74)	2.18 _0.12 (85)	+11.8
4.0	−60	1.J9±0.14 (50)	1.81±0.13 (50)	− 4.2
5.2	30	1.85±0.10 (52)	1.89±0.12 (49)	+ _2
5.5	30	2.00±0.30 (112)	0.96±0.14 (114)	−52
5.0	30	0.59±0.05 (80)	0.48±0.06 (65)	−18.6
5.0	10	1.16±0.11 (90)	0.75±0.05 (54)	−35.3

the MAP to 70-90 mmHg over a period of [illegible]. After compensation all animals main[tained] a stable MAP throughout the observation [period].

[Tow]ards the end of the bleeding a complete cir[culator]y standstill occurred in the tenuissimus mus[cle. Th]is arrest period usually lasted 5-20 min [a]fter flow gradually reappeared. Capillary [dis]tribution was evident from the start and 50% of the capillaries did not resume blood [flow]. The non-perfused capillaries were usually between perfused capillaries, i.e. capillaries [s]een without circulation despite flow in both [feedin]g and draining vessels. Since these capillaries [onl]y contained only a few RBC's it was only with [hel]p of the video tape recordings and the draw[ings] made before bleeding that they could be de[lin]eated and subjected to careful observation along their entire course. In no instance were these capillaries seen to be collapsed and the RBCs could often be seen to oscillate slightly in the lumen. However a significant observation was that all of the non-perfused capillaries that were possible to observe contained one or several WBCs somewhere in the lumen (Fig. 3). Due to their low contrast the WBCs easily escaped the observer's eye at first scrutiny of the capillaries. Typically the WBCs were found lodged at the bulgings of endothelial cell nuclei or at capillary branchings (Fig. 3*b*). There were no indications that the WBCs were halted by adhesion to the capillary walls since they often slided smoothly back and forth in front of the hindrance. In some cases plugging WBCs were seen to be suddenly dislodged from a capillary whereupon the capillary

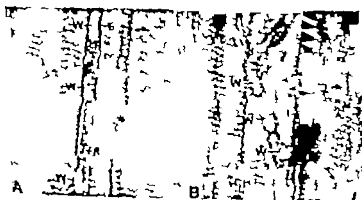


Fig. 3. In vivo photographs of WBC plugging of skeletal muscle capillaries in shock. (A) Asterisk, empty capillary. *W*, white blood cells, *R*, red blood cells, *E*, endothelial cell bulging. (B) WBC plugging at capillary branching. *W*, white blood cells, *R*, red blood cells. Arrows with bars, blocked capillaries. Arrow with asterisk indicates direction of [interm]ittent flow.

was instantaneously reperfused This phenomenon clearly illustrated the plugging effect of the WBCs

Platelet or RBC platelet aggregates were not seen in the microvasculature Consequently capillary plugging by these blood corpuscles could not be demonstrated in the skeletal muscle microcirculation

In vitro experiments

The MAP response to bleeding in the animals used as blood donors was principally similar to that of the in vivo series

The deformability data for the WBCs before and after shock are presented in Table 1 MDT values for WBCs in shock show great variation in relation to pre shock values The mean percentage change in MDT is −16±9 9 (S E) Statistically this figure is not significantly separated from zero when tested according to Wilcoxon s ranking test or Student s *t* test By analysing the distribution of all DT values it seems that the scatter in MDT change is largely dependent on the number of particularly stiff cells possibly lymphocytes (Bagge & Brånemark 1977) which happened to be present in each sample If the stiffer cells (DT ranging from 3 to 15 s) are excluded from the calculations the MDT change becomes smaller but there is still no consistent direction of change between pre-shock and shock values

The plasma viscosity expressed as particle velocity did not differ significantly between the samples within each experiment

DISCUSSION

This investigation provides evidence of WBC plugging as an important cause of capillary flow maldistribution in skeletal muscle in shock WBC plugging of capillaries has been observed in many tissues and species including man (cf Bagge & Brånemark 1977) However normally the plugging is only occasional and of short duration (0.5–3 s) The phenomenon is obviously related to the size spherical shape and relatively high viscosity of the WBCs (Bagge et al 1977*a*)

Passage of WBCs through the capillaries in skeletal muscle which are long and narrow (Eriksson & Myrhage 1972 Myrhage & Hudlická 1976) requires considerable deformations of the WBCs In early shock, the perfusion pressure is substantially reduced in the skeletal mu[illegible] crocirculation (Mellander & Lewis [illegible] theoretical grounds it could therefore be ex[illegible] that WBC plugging should occur more freq[illegible] and also be of longer duration in shock tha[illegible] normal pressure conditions Such a conclu[illegible] supported by the present finding of frequen[illegible] plugging in the tenuissimus muscle capillaries [illegible] lar observations of WBC plugging in hear[illegible] shock have also been reported by Wilson (19[illegible] histological and electron microscopic studies [illegible] lungs in dogs Wilson found a 65% increase [illegible] number of WBCs trapped in alveolar capillar[illegible] shock compared to controls Further Wilson [illegible] in both in vivo studies and histological section[illegible] the WBCs tended to become larger and [illegible] spherical during shock Theoretically such [illegible] the WBCs would lead to a reduced deformabil[illegible] consumption of some of the membrane area [illegible] ble for deformation (cf Bagge et al 1977*a* B[illegible] al 1977*b*) However the present studies in vi[illegible] not indicate that decreased WBC deformability [illegible] curs in shock It should be emphasized th[illegible] increased frequency of WBC capillary plu[illegible] does not require a decrease in the deformabil[illegible] the WBCs per se but can be explained by [illegible] driving pressure alone

The redistribution of blood flow in hemor[illegible] shock tends to maintain a normal perfusion [illegible] organs such as heart brain and kidneys, whil[illegible] perfusion of peripheral tissues such as the sk[illegible] muscle is markedly decreased due to vasocon[illegible] tion The resultant reduction in capillary perf[illegible] pressure would make the microvasculature [illegible] skeletal muscle more susceptible to WBC plu[illegible] than that of organs with a relatively higher pre[illegible] level In fact histologic studies in shocked [illegible] have failed to reveal any substantial WBC e[illegible] ment in other organs than the lung (Wilson 19[illegible]

Baeckström et al (1971) found evidence for [illegible]croplugging of the skeletal muscle vascula[illegible] after a severe hemorrhage using the isolated [illegible] limb preparation in cat These investigators [illegible] gested that the plugs were formed by platel[illegible] RBC aggregates A normal pressure-flow rela[illegible] ship could be gradually restored by high pres[illegible] perfusion with a cell free medium and massag[illegible] the preparation In our opinion such an effect [illegible] equally well be explained by WBC plugging [illegible] platelet or RBC aggretages

The preliminary in vitr[illegible] [illegible]ses dem[illegible]

qualitative alteration of WBC deformability ...ck. Despite this statistical insignificance, ... there seems to be a tendency of the MDT ... case somewhat in shock. A tentative explanation could be that the stiffer cells within the nor... population would be more frequently trapped in ... microvasculature in a low pressure situation. To ... this theory two of the present experi... were extended to include also analysis of the ... WBCs collected from the hind leg muscula... (the deep femoral vein). The results of ... analyses showed a more pronounced decrease ... MDT compared with pre-shock values, than ... BCs from central venous blood in shock. The ...ation of the DT values in the samples from ...moral vein indicates that the skeletal muscle ... vasculature is more prone to act as a filter for ... deformable WBCs than other vascular cir... This hypothesis, however will require fur... investigation also involving simultaneous blood ... ing from the local artery and vein supplying ... ent organs.

... observation of WBC plugging as a major ... of capillary flow maldistribution in skeletal ... le in hemorrhagic shock is striking. The in ... expts. indicate that this is primarily a pres... related phenomenon. It can be argued, how... that the vital microscopic observations do ... reflect a generalized phenomenon since they ... restricted to a small part of the musculature ... the observations point to a principally impor... phenomenon which certainly deserves further ...tion.

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rtid baroreflex heart rate control during the active the assisted breathing cycle in man

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MELCHER, A., Carotid baroreflex heart rate control during the active and the assisted breathing cycle in man Acta Physiol Scand 1980 108. 165-171 Received 9 May 1979 ISSN 0001-6772. Departments of Clinical Physiology Karolinska Hospital and Serafimer Hospital, Stockholm, Sweden.

The interaction between the phase of the breathing cycle and the carotid baroreflex heart rate control was studied in fifteen healthy subjects with special reference to respiratory sinus arrhythmia (RSA). Carotid baroreceptors were stimulated by neck suction. With a standardized breathing pattern, two types of experiment were performed. (A) Neck suction applied in counter phase with the respiratory arterial pressure variations, this reduced the amplitude and shifted the phase of the transmural carotid arterial pressure oscillations but did not influence the RSA. (B) During active as well as ventilator assisted breathing, brief periods of neck suction were applied during either inspiration (insufflation) or expiration. Neck suction during active inspiration did not change the amplitude of the RSA but when applied during expiration, increased it by 6.8 beats × min⁻¹ ($P<0.001$). In the ventilator experiments, neck suction during insufflation and expiration augmented the RSA equally by 5.3 beats × min⁻¹ ($P<0.05$). The results indicate a decreased sensitivity of the carotid arterial baroreflex during active inspiration, with no equivalent during assisted breathing, and suggest that the change in baroreflex sensibility is prerequisite for the rise of the RSA.

Key words Arterial baroreflex cardiac reflexes, heart rate regulation, intermittent positive pressure ventilation, respiration sinus arrhythmia

nnection between the phases of the breathing e and heart rate is well known in laboratory als and man During quiet breathing it appears e respiratory sinus arrhythmia (RSA), i.e. an atory acceleration of the heart rate and a leration during expiration. The arrhythmia been attributed to several mechanisms. Early ies (for review see Heymans & Neil 1958) indi- d that the RSA was mediated by a pulmonary- iac reflex activated by the expansion of the during inspiration. Other animal experiments wed that the RSA could be due to an influence he respiratory centre (and higher centres) within central nervous system on the cardiovascular tre in the brain stem. Bainbridge (1920) how r suggested that the inspiratory tachycardia was sed by a heart acceleration reflex elicited by reased diastolic filling of the heart during inspira- n. Finally the systemic arterial baroreflex has been implicated in the genesis of RSA (Matthes & Ebeling 1948 Mechelke 1953) It was claimed that the RSA was secondary to the respiratory oscillations of the arterial pressure which activated the baroreflex.

In a recent study of the central hemodynamics and RSA in man (Freyschuss & Melcher 1976) the heart rate during the breathing cycle displayed a positive correlation, beat-by-beat, to the systemic arterial pressure One is therefore inclined to question the systemic arterial baroreflex as a cause of the arrhythmia, since this would be expected to yield a negative correlation, reflecting its depressor effect. However the baroreflex may be involved in another manner animal experiments have demonstrated that the sensitivity of the reflex may change during the respiratory cycle (Koepchen et al. 1961 Haymet & McCloskey 1975 Davidson et al 1976) Thus a lowered sensitivity during inspira-

tion would allow a simultaneous rise in heart rate and arterial pressure a drop in rate during expiration in spite of falling pressure could be explained by a restored reflex sensitivity Experiments in man have supported this view (Melcher 1976 Eckberg & Orshan 1977)

It is not known what mechanisms might cause an inspiratory inhibition of the arterial baroreflex However it has been shown that the baroreflex sensitivity can be influenced by the activity of higher nervous centres (see Smith 1974) and by the interaction with the afferent nervous inflow from cardiopulmonary receptors (Castenfors & Sjöstrand 1973 Vatner et al 1975 Koike et al 1975 Bevegård et al 1977) The latter findings have led to the hypothesis that the increased diastolic filling of the heart during inspiration activates a cardiac reflex which inhibits the arterial baroreflex (Melcher 1976) If so inhibition would not occur during insufflation of the lungs when cardiac filling is impaired

The present study was performed (*a*) to test whether or not the respiratory oscillations of the arterial pressure can induce the RSA via the carotid sinus baroreflex and (*b*) to investigate if there is a change in baroreflex heart rate control in phase with respiration in conscious man and whether this persists during intermittent positive pressure ventilation Part of this work has been presented before (Melcher 1976)

MATERIAL AND METHODS

15 healthy male volunteers participated in the study Their mean age was 27 7 years (range 1 37) The investigation had been approved by the Ethical Committee of the Karolinska Institute

The subjects breathed sinusoidally in pace with a beam on an oscilloscope at a rate of 6 cxmin This rate was chosen to produce an optimal sinus arrhythmia (Angelone & Coulter 1964).

The baroreceptors of the carotid artery were stimulated by applying subatmospheric pres ure to the neck (P_{ns}) thereby increasing the transmural arterial pressure (Ernsting & Parry 1957 Bevegård & Shepherd 1966) The equipment used comprised a stiff wide collar connected to a vacuum pump The collar extended cranially to the ear lobes and was supported at its upper edge laterally by the mandible and dorsally by the processus mastoideus and occipital bone Against the thorax it rested on the clavicles and the ventral part of the upper thoracic aperture The edges were lined with foam rubber The pressure within the collar was regulated by the effect of the pump It could be varied either slowly by changing the effect of the pump or in an approximately square wave fashion by using a stop-cock between the collar pump The collar was equipped with one pressure transducer for monitoring of P_{ns} and another arterial pressure transducer in order to measure the pressure differentially against the pressure in neck thus equalling the *transmural pressure of the artery* (Pca) Monitoring of the esophageal pressure subject showed no transmittance of P_{ns} to intrathoracic pressure

Tidal volume (V_T) was measured by pneumotachography The subjects breathed through a mouthpiece the airflow was sensed by a Fleisch flow head and a differential pressure transducer (EMT 3.. Siemens-Elema Sweden) V_T was obtained from the flow by electrical integration The system was volume calibrated against a water spirometer or a precision pump (Norlander 1966) immediately before and after each experiment V_T is given in liters ATPS

Intermittent positive pressure ventilation (IPPV) was performed with a respirator (Servo ventilator Siemens-Elema) with a respiratory frequency 6 min⁻¹ and a tidal volume of 1.5 l The ventilator was specially designed so the insufflation phase could be extended to comprise 50% of the respiratory cycle end-inspiratory pressure (Pei) was obtained from the ventilator and recorded together with the other variables

Intrathoracic pressure was recorded in one subject using the ordinary oesophageal balloon technique

Heart rate (HR) was counted from ECG leads either by a heart rate meter (Ott Elektromedizin) or by hand from a recording (paper speed 50 mm s⁻¹)

Pressures were measured with capacitive transducers (EMT 35 and 34 Siemens-Elema Sweden)

The variables were recorded on an ink jet recorder (Mingograf 81 Siemens-Elema Sweden)

Procedures

The experiments were performed with the subject in supine position In expt (A) (see below) a short catheter was inserted percutaneously into a brachial artery for pressure monitoring Before the actual experiments started intermittent neck suction of varying degree was applied in order to accustom the subjects to the sensation of negative pressure They also practised breathing with the respirator and no measurements were begun before subjects were able to relax and could avoid active respiration IPPV recordings were accepted only if the respiratory pressure rose steadily during insufflation

Two series of experiments were performed (A) subject moderate neck suction synchronized with respiration was applied sinusoidally in counter phase to the oscillation of the systolic arterial pressure so as to minimize and to shift the phase of the transmural pressure variations in the carotid artery (counter stimulation) Neck suction was adjusted manually in pace with the subject s respiration on 5 occasions and automatically controlled in 4 subjects. (B) In 15 subjects square wave neck suction was applied for approximately 3 s during either inspiration or expiration 4 expt however had to be discarded since the subjects spontaneously increased their V_T by more than 25% when neck suction was applied

nspiration. Thus, the results from 11 subjects are
f

l experiments the subjects were instructed to
in pace with the oscilloscope beam with raised
erwise spontaneously chosen V (except for the
or expt). In between periods of measurements the
were disconnected from the mouth piece

ations

mal values of all variables were calculated as means
t 6 (usually 5) consecutive breaths. The following
were extracted tidal volume (V), maximum and
um heart rate (HR_{max} and HR_{min}), amplitude of the
(HR_{max}–HR_{min}), maximum and minimum carotid
transmural systolic pressure (Pca_s) and its
ode (Pca_s only in expts (A)) the time for oc-
ce of these peak values during the respiratory cycle
tion to the start of inspiration (defined from the
tachograph tracing) and, finally the subatmos-
stimulation pressure in the neck collar
alations were performed on systolic arterial pres-
s this has shown good correlation to heart rate in
of the arterial baroreflex function (Smyth et al.

effects of the carotid sinus baroreceptor stimulation
been tested with the paired *t*-test, levels of signifi-
$P<0.05$ (*), $P<0.01$ (**) and $P<0.001$ (***).

ULTS

ffect of carotid artery counter
lation (CS)

s are summarized in Fig. 1 and Table 1 Neck
sion during CS varied between 0 and −10.1
Hg. By adjusting the nadir of P_{max} to early inspi-
t it was possible to reduce the ordinary res-
tory Pca_s oscillations by 27%. During control
thing the Pca_s varied between 124 and 112
Hg and during CS between 127 and 119 mmHg.
appears from Table 1 and Fig. 1 the occurrence
maximum and minimum Pca_s were delayed

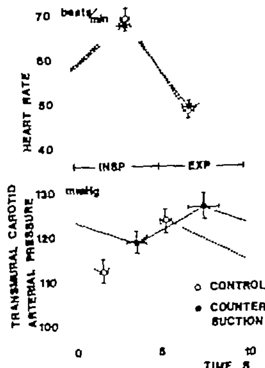


Fig. 1 Average (±S.E.) peak heart rates and systolic transmural carotid arterial pressure during the breathing cycle (=9). Lines between peak values do not represent true time course

and the phase relation between Pca_s and re spiration was changed In 4 subjects the ordinary pressure oscillations were almost reversed in relation to breathing by this stimulation pattern. The RSA however was not affected neither in its amplitude and peak values nor in its time course during the breathing cycle (Table 1 Fig. 1)

le 1 *Effect (mea ± S.E.) of carotid artery counter stimulation (CS) on tidal volume (V_T) iration sinus arrhythmia (RSA) carotid arterial transmural systolic pressure (Pca_s) and time for peak es of heart rat (HR) nd Pc s(n=9)*

	V (l ATPS)	Amplitude of RSA beats min⁻¹)	Amplitude of Pca_s (mmHg)	Time after start of insp HR_{max} (sec)	Time after start of insp HR_{min} (sec)	Time after start of insp $Pca_{s\,max}$ (sec)	Time after start of insp $Pca_{s\,min}$ (sec)
ntrol	1 92±0.13	21 1±2.0	11 3±0.5	3.3±0.2	6.7±0.3	5.3±0 3	1.6±0 1
	1 96 0 13	19 6 2.3	8.2±0.9	3.2±0.3	6.8±0.4	7.6±0 9	3 6±0 7
fference	0 04+0 06	1.5±0 6	3 1±0.9	0.1 0.3	0.1_0.2	2.2±0 7	2.0±0.7
p	s.		$P<0.01$	s.	s.	$P<0.05$	$P<0.05$

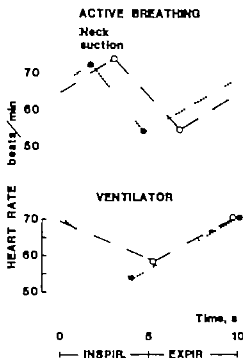


Fig 2 Effect of inspiratory neck suction on peak values of heart rate during active and ventilator assisted breathing Means from eleven subjects Circles represent control and dots neck suction experiments Lines between peak values do not represent true time course

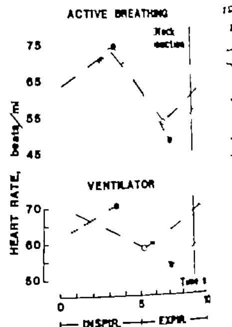


Fig 3 Effects of expiratory neck suction on peak v of heart rate during active and ventilator assisted b ing Means from eleven subjects. Symbols as in Fig

The effects of inspiratory and expiratory carotid sinus baroreceptor stimulation during active and ventilator assisted breathing

Results are presented in Figs 2 and 3 and Table 2. During active breathing the mean P_{stim} was -27 3 mmHg and in the ventilator expts it averaged -26 7 mmHg. Individually identical P_{stim} were used during inspiration and expiration stimulation averaged 14 7 cm of water in the ventilator expts It was not affected significantly by the suction

During active breathing *inspiratory* and influenced neither the amplitude of the RSA or HR_{max} or HR_{min} but peak values of heart rate the breathing cycle occurred significantly earlier the ventilator control expts. with IPPV the appeared as a drop in heart rate during insuffl

Table 2 *Mean (±S E) control values and effects of inspiratory (IS) and expiratory (ES) barore stimulation by neck suction during active and ventilator assisted breathing (n=11)*

	V (1 ATPS)	RSA ampl (beats/min)	Time (s) from start if insp HR_{max}	HR_{min}
Active breathing				
Control	1.86±0 19	19 9±2.0	3 02±0..4	6.65±0.35
IS	+0 10±0 07	−1 4±0.9	−1 36±0.28*	−2.06±0.49*
ES	−0.07±0 07	+6.8±1 4	+0.18±0.36	+0 45±0..1
Ventilator breathing				
Control	1.50±0 00	1 0±1 4	9 68±0 44	5 19±0.40
IS	0 00±0.00	+5.3±2 1	+0 4 ±0 44	−1 17±0.54
ES	0 00±0.00	+5.3±1 7*	−6.25±0 9*	+1 90±0 41

+ denotes later and − earlier during the respiratory cycle
*=P<0 05 **=P<0 01 and ***=P<0.001

› acceleration of the rate during expiration 2 and 3). Inspiratory neck suction during nduced a more marked arrhythmia compared trols (Table ?) due to a lower HR_{min} at the insufflation, while HR_{max} was not affected. as the time course of the arrhythmia changed ratory stimulation during IPPV

neck suction applied during expiration the uring active breathing was augmented (Table appears from Fig. 3 this is because HR_{min} expiration was lowered by 6.1 beats per min 001) The HR_{max} was not affected and the or occurrence of peak heart rate values during spiratory cycle was unchanged. In the IPPV baroreceptor stimulation during expiration nted the arrhythmia, again by further de- ng minimum heart rate by 5.1 beats×min⁻¹ 001) There was a marked change of the heart me course compared to the IPPV control ($P<0.001$ Fig. 3)

USSION

technique of stimulating the baroreceptors of arotid artery by exposing the neck to reduced ure has been used previously (Ernsting & Par 57 Bevegård & Shepherd 1966 Theron et al.). In the present study (series A) the systemic tal pressure was measured differentially ist the subatmospheric pressure to obtain a di- measure of the transmural pressure across the of the carotid artery A prerequisite is how that the reduced pressure is transmitted un- ged in the tissues surrounding the vessel. ever this can be assumed for several reasons on et al. (1967) Shubrooks (1972) Coles (for see Wolthuis et al. 1974) and Eckberg (1976) ied the technique extensively and found a 90% smission of the reduced pressure in the tissue sophagus and in the internal jugular vein. The cent findings of Ludbrook et al. of a 64% nsmission of −60 mmHg is difficult to assess e their technique is only briefly described e.g. m measuring the transmission to the internal lar vein The pressure drop in the vein may be lerestimated if not measured immediately at the et of neck suction before inflow of blood from er parts of the venous system has partly restored pressure (Eckberg 1976). The only explanation incomplete transmission would be a change in volume due to shift of blood with some disten- tion of the tissues. With slightly reduced pressure (e.g. −10 mmHg) the change in volume is small and the compliance of the neck tissues are comparatively high, i.e. the pressure is well transmitted. Thus it seems justified to use neck suction quantitatively in the present experiments, series A In the second part of the study series B where reduced pressures in the order of −30 mmHg are used the question of pressure transmission is not crucial since the responses to identical degree of neck suction during inspiration and expiration are compared

The time course and the amplitude of the RSA in the present work are in accordance with previous results (Freyschuss & Melcher 1975) during both active breathing and IPPV The completely unaffected RSA when CS changed the respiratory variations of Pca shows that this arrhythmia cannot be explained by the ordinary negative feedback of the carotid baroreflex. Together with the ability of the baroreceptor stimulation to increase the RSA only when applied during expiration this clearly speaks in favour of the hypothesis that there is an inspiratory inhibition of the arterial baroreflex. This means that RSA would occur even in the absence of arterial pressure variations. Further evidence against the assumption that the baroreflex generates the RSA is found in the (B) expts the effect of expiratory neck suction (lowered HR_{min}) appears while the stimulus is still in progress, while there is no effect on HR_{max} which falls during inspiration showing that there is no off effect when suction is released. Consequently a rising arterial pressure during inspiration is not coupled to the slowing of the heart rate during expiration and a fall in pressure during expiration is not responsible for the tachycardia during the ensuing inspiration. The rapid appearance of the bradycardia response is in accordance with a reflex response time of less than one second (Eckberg 1976) The results of expts. (B) are broadly in accordance with those of Eckberg & Orshan (1977). However the present study seems somewhat more clear-cut in showing no effect of inspiratory stimulation on RSA amplitude, while they found some pulse interval prolongation. This difference may be due to differences in experimental design. Their subjects breathed at a higher unstandardized rate and with smaller V and the stimuli were of shorter duration, 0.6 s. Thus there was a more powerful inhibition of the baroreflex in my expts., possibly due to larger breaths. On the other hand it may be noted that even in the present

expts the inhibition was not complete since inspiratory stimulus was able to change the time course of the RSA The ventilator expts. were undertaken to elucidate the possible interference of reflexes from cardio-pulmonary receptors and/or the central nervous respiratory drive with the arterial baroreflex The Pei were lower than during general anaesthesia (Hedenstierna & Löfström 1972) indicating that the subjects were unable to relax completely but still enough to abolish the inspiratory drop in pleural pressure In similar expts this has been shown to impede the filling of the heart during inspiration (Freyschuss & Melcher 1976) The equal effects on HR of inspiratory and expiratory suction during IPPV show that there is in contrast to active breathing no inspiratory inhibition of the baroreflex Thus lung expansion with 1.5 l does not depress the baroreflex function and the inspiratory inhibition is not of pulmonary origin This is in keeping with recent canine expts which show that no inhibition of the baroreflex occurs during prolonged periods of inflation or slow insufflation of the lungs (Haymet & McCloskey 1975 Gandevia et al 1978)

Since there was a voluntary suppression of the nervous inspiratory activity during IPPV the difference from active breathing could be explained by a central nervous mechanism The cortico-hypothalamic influence upon the baroreflex function e g during the "defence reaction muscular exercise and during sleep and anesthesia has been shown in several works (for refs see Smith 1974) This explanation would also be in accordance with studies of the RSA in animals (Anrep et al 1936 Joels & Samueloff 1956 Levy et al 1966 Davidson et al 1976 Gandevia et al 1978) demonstrating a coupling between the central nervous respiratory drive and the RSA In a previous study on man however no evidence was found for a central nervous mechanism generating the RSA (Freyschuss & Melcher 1975)

An alternative explanation would be that the baroreflex inhibition is associated with the diastolic filling of the heart the classical Bainbridge effect There is now experimental evidence that tachycardia can be induced from certain areas of the atria (for refs see Linden 1975) and in some animal expts the elevated heart rate is explained by an inhibitory influence of cardiac afferent impulses on the arterial baroreflex (Castenfors & Sjöstrand 1973 Koike et al 1975 Vatner et al 1975) There are observations indicating that this [illegible] also for man although the results are [illegible] equivocal (Bevegård et al 1977 Takeshita [illegible] 1979) In the present study inhibition [illegible] baroreflex was seen only when there were [illegible] requisites for increased filling of the heart [illegible] ing active inspiration

In conclusion the present results show [illegible] RSA in man is independent of the respiratory [illegible] chronous arterial pressure variations and the arrhythmia at least partly is due to an inh[illegible] the arterial baroreflex heart rate control [illegible] with respiration This inhibitory influence [illegible] pendent of volume expansion of the lung [illegible] associated to active inspiration with increased [illegible] nous return to the heart Thus it is possibly [illegible] from the central nervous system or receptors [illegible] the heart

This study was supported by grants from Karol[illegible] stitutet The respirator was kindly put at my disposal [illegible] Siemens-Elema Ltd Sweden.

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...ective tissue of 'fast' and 'slow' skeletal muscle ...ts—effects of endurance training

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KOVANEN V, SUOMINEN H & HEIKKINEN E. Connective tissue of "fast" and "slow" skeletal muscle in rats—effects of endurance training. Acta Physiol Scand 1980, 108: 173–180. Received 9 May 1979. ISSN 0001-6772. Department of Public Health, University of Jyväskylä and Department of Public Health Sciences, University of Tampere, Finland.

The connective tissue of two skeletal muscles having different contractile properties was investigated in trained and untrained rats. The animals to be trained were put to run on a treadmill 5 days a week for 4 weeks. The "slow" m. soleus (MS) showed higher malate dehydrogenase activity but lower lactate dehydrogenase activity compared to the "fast" m. rectus femoris (MRF). When whole muscles were taken into account, the concentrations of both hydroxyproline and hexosamines were higher for MS compared to MRF. In the middle section of MS there were more hexosamines than in that of MRF, but no similar difference existed in hydroxyproline. The histochemical staining of collagen, however, suggested that there is also more internal collagen for MS as against MRF. It can be supposed that collagen of MRF and MS is differently distributed in different muscle connective tissue components. Compared to MS, the solubility of collagen was higher in MRF, whereas no significant difference between the muscles existed in the prolyl hydroxylase activity. The concentrations of hydroxyproline and hexosamines or the solubility of collagen were not affected by the training given, but the activity of prolyl hydroxylase was increased in MS suggesting that the metabolism of collagen may be accelerated by physical training.

Key words: Collagen, glycosaminoglycans, prolyl hydroxylase, fast/slow skeletal muscle, endurance training, rat

...ew of the magnitude and intensity of connec... ...tissue research in both basic and applied areas ...little is known about the connective tissue of ...tal muscle, particularly as to the effects of ...ical exercise and training. Physical training is ...n to affect both the physical and chemical ...erties of several other connective tissues ...th & Gould 1975, Ingelmark 1945, 1948, Tipton ...l. 1967, 1970, 1975, Viidik 1967, 1968). As to ...etal muscle, previous animal experiments have ...n that there exists an obvious connection be... ...n the synthesis of the components of muscle ...le and muscle connective tissue both in hyper... ...hied skeletal (Turto et al. 1974) and heart ...dy et al. 1972) muscles. According to Jablecki ...l. (1973) the activation of connective tissue cells ...n important prerequisite to work-induced mus... ...ar growth.

The present investigation was undertaken to study the connective tissue of "fast" and "slow" skeletal muscles with special reference to the effects of endurance-type physical training. For this purpose the concentrations of collagen and glycosaminoglycans as well as the solubility of collagen and the activity of prolyl hydroxylase, i.e. an enzyme participating in collagen biosynthesis were determined.

MATERIAL AND METHODS

Animals and training procedures

Three-month-old male Wistar rats (n=25) were randomly assigned to test and control groups. The animals to be trained were gradually adapted to running on a treadmill. The training was performed on an 8° inclined motor driven treadmill at a speed of 24 m/min. The rats ran 5 days a week for 4 weeks, the daily exercise being per

formed in two sessions the first in the morning and the second in the afternoon The training time was increased weekly from 2×10 min in the first week to 2×40 min in the last week

Both groups were housed in 33×21×18 cm cages two animals to each cage All animals lived in normal laboratory conditions (humidity temperature and the length of day were kept constant) and commercial pellet food (Hankkija Finland) and water were given ad libitum

Muscle samples

After the training period the trained and control rats were killed by decapitation and blood samples were taken for the determination of hemoglobin (cyanmethemoglobin method) Heart m rectus femoris (MRF) and m soleus (MS) were excised The hearts were opened immersed several times in 0.9% NaCl to remove blood dried slightly on a filter paper and weighed After two days of lyophilization the dry weights of the hearts were also determined MRF and MS were horizontally cut into three sections of equal length The muscles of both hind limbs were used in the analyses in order to get as large a number of observations as possible The samples for histochemistry were immediately taken for handling as described below whereas the other muscle samples were frozen and stored at −80°C for further analyses

Histochemical analyses

The histochemical analyses were performed on the middle section of the muscles The samples for the histochemical determination of collagen were first kept in a cold (+4°C) Ringer solution and then fixed in Baker s formalin The samples were handled routinely with the paraffin technique and stained with a modified Herovici staining procedure (see Herovici 1963)

The muscle samples for the myofibrillar ATPase were vertically embedded in OCT-compound (Tissue Tek Ames Co) rapidly frozen in isopentane prechilled with liquid nitrogen and stored at −80°C Sections were cut with a cryostat microtome (Ames II) at −20°C and stained for myofibrillar ATPase (Padykula & Herman 1955) The fibers were identified as slow twitch (ST) or fast twitch (FT) on the basis of the ATPase activity as described by Gollnick et al (197?) The results were expressed as a percentage of ST fibers

Biochemical analys s

Enzymes of energy metabolism After thawing a sample of the middle section of muscle (about 60 mg from MRF and 20 mg from MS) was homogenized 12 times for 3 s in 50 volumes of ice-cold 10 mM K-phosphate buffer (pH 7 4) containing 1 0 mM EDTA and 0 1% Triton X 100 by using a Potter Elvehjem homogenizer kept in an ice bath A sample of the homogenate was diluted and the activities of malate dehydrogenase (MDH) and lactate dehydrogenase (LDH) were determined (Biochemica Test Combination, Boehringer Mannheim) The reaction rates were measured at +_5°C with a Unicam SP 1800 spectrophotometer connected to a Unicam AR _5 recorder The enzyme activities were expressed as micromoles NADH oxidized per minute per g wet weight of muscle

Hexosamines The samples of proximal [illegible] distal muscle were lyophilized, weighed, and h[illegible] in ? M HCl for 16 h at +103°C An aliquot of the [illegible] lyzate was adsorbed on a cationic exchange [illegible] 50 H) and eluted with M HCl (Boas 1953 [illegible] centration of hexosamines (Gatt & Berman [illegible] used as a measure of glycosaminoglycan [illegible] muscle

Hydroxyproline and nitrogen The remainder [illegible] hexosamine hydrolyzate was further hydrolyzed [illegible] HCl for 16 h at +120°C and the concentrations of [illegible]xyproline (Kivirikko et al 1967) and nitrogen [illegible] & Zilversmit 1963) were assayed to indicate the [illegible] of collagen and total protein respectively

Solubility of collagen and total protein A sample [illegible] middle section of muscle was homogenized three [illegible] 3 s in 4 ml of ice-cold 0 45 M NaCl by using a [illegible] Elvehjem apparatus kept in an ice bath. The [illegible] was agitated in a swirling shaker for 24 h at +4°C [illegible] centrifuged at 32 000 × g for 60 min further at +4°C [illegible] extraction procedure was repeated twice [illegible] 1968) Both the sediment and combined supernatants [illegible] evaporated to dryness and hydrolyzed in 6 M [illegible] 16 h at +120°C From the hydrolyzates, the con[illegible] tions of hydroxyproline (Kivirikko et al. 1967) and [illegible] gen (Minari & Zilversmit 1963) were determined [illegible] percentage of supernatant hydroxyproline from the [illegible] (sediment + supernatant) hydroxyproline was taken [illegible] measure of collagen soluble in 0 45 M NaCl [illegible] the nitrogen analyses were used to estimate total [illegible] protein

Prolyl hydroxylase (PH). After thawing, a sample [illegible] middle section of muscle (about 100 mg from MRF [illegible] 30 mg from MS) was homogenized twelve times [illegible] in 30 volumes of ice-cold 0 02 M Tris-HCl buffer [illegible] containing 0 1 M KCl and 0 1% Triton X 100 by [illegible] Potter Elvehjem homogenizer kept in an ice-bath [illegible] homogenate was centrifuged at 15 000 × g for [illegible] +4°C For the assay of PH activity 130 µl of the [illegible] natant fluid was incubated with C-proline labelled [illegible] collagen substrate (45 000 dpm a gift from Prof [illegible] Kivirikko) 50 mM Tris HCl buffer (pH 7.8 at [illegible] 2 mM ascorbic acid 0.5 mM α-ketoglutarate, [illegible] FeSO and mg/ml albumin in a final [illegible] (Kivirikko & Prockop 1967) After 60 min of [illegible] at +37°C the samples were hydrolyzed in 6 M HC[illegible] 16 h at +120°C and total C-radioactivity (Prockop [illegible] Ebert 1963) as well as C-hydroxyproline (Juva & P[illegible]kop 1966) were assayed in the hydrolyzate The [illegible] activity was counted with an LKB Wallac [illegible] scintillation counter using an external standard [illegible] ratio method for dpm calculation The PH activ[illegible] expressed as dpm C-hydroxyproline formed [illegible] wet weight of muscle per mg sediment hydroxypro[illegible]line as well as per mg supernatant protein For the [illegible] of hydroxyproline the 15 000 × g sediment [illegible] lyzed in 6 M HCl for 16 h at +1?0°C From the [illegible] lyzate hydroxyproline was determined according [illegible] Kivirikko et al (1967) The concentration of [illegible] hydroxyproline was used as the measure of conn[illegible] tissue in the muscle samples Protein was determined [illegible] the 15 000 × g supernatant [illegible]dified method

Physical characteristics of trained and control rats

S.D. and number of observations are given

	Trained rats	Control rats	P
body weight (g)	318±15 (12)	318±15 (13)	n.s.
body weight (g)	367±21 (12)	400±31 (13)	<0.01
ght of heart (mg)	1 110±61 (12)	1 009±71 (13)	<0.01
ght of heart (mg)	257±14 (12)	232±17 (13)	<0.001
ght of MRF (mg)	1 037±73 (9)	1 064±96 (10)	n.s.
ght of MRF (mg)	261±24 (9)	269±25 (10)	n.s.
ght of MS (mg)	134±19 (9)	137±12 (10)	n.s.
ght of MS (mg)	35± 5 (9)	32± 3 (10)	n.s.

et al. (1951). T ensure that adequate amounts of protein were used in the determinations of the very different volumes of the 15 000 g super of the muscle homogenates were assayed as a experiment.

al methods

d procedures were used to calculate means, standard deviations (S.D.) and standard errors (S.E.). The al significances ere calculated by Student's *t*-test non-correlating means.

ULTS

e 1 shows some of the physical characteristics the trained and control rats. Compared to the ol group the trained rats had lower final body t but higher heart wet and dry weights. The al muscle weights did not, however significantly differ between the trained and untrained ts. The blood hemoglobin of the trained rats 5 ±7 g 1 and that of the untrained 154±6

The muscle fiber composition and the activities e enzymes of energy metabolism are shown in Table 2. The percentage number of slow twitch fibers in MS was about 90% whereas that in MRF was only 1 to 9%. The trained animals showed lower LDH activity compared to the control group the difference being significant in MRF. There were no significant differences between the trained and untrained rats in MDH activity although it tended to be higher in both muscles of the trained rats.

No histopathological alterations were observed in the specimens obtained from the trained and control animals. Generally it was noticed that collagen tained more strongly in the perifascicular space i.e. perimysium than around the individual muscle fibers i.e. endomysium. Furthermore somewhat more collagen was observed in MS than in MRF (Fig. 1).

When the whole muscles were taken into account, i.e. when the results from the proximal, middle and distal muscles were combined (Table 3) the hydroxyproline and hexosamine concentrations were significantly higher for MS compared to MRF whereas the nitrogen concentration tended to be higher for MRF. The hydroxyproline/nitrogen ratio

Table 2. *Fiber composition and enzyme activities of m. rectus femoris and m. soleus in trained and control*

S.D. and number of observations are given

Variable	Muscle	Trained rats	Control rats	P
fibers (%)	MRF	1.2±1.0 (6)	1.5±1.7 (6)	n.s.
	MS	91.0±5.9 (6)	88.3±4.9 (6)	n.s.
LDH (µmol g min)	MRF	650±43 (9)	745±47 (10)	<0.001
	MS	205±43 (9)	238±30 (10)	n.s.
MDH (µmol g min)	MRF	479±60 (9)	424±67 (10)	n.s.
	MS	752±140 (9)	674 95 (10)	n.s.

There was significant difference (P<0.001) between MRF and MS in all variables.

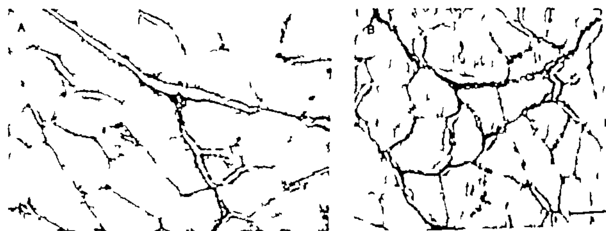


Fig 1 Cross sections of muscle fibers from m rectus femoris (A) and m. soleus (B) stained according to the F[illegible] staining procedure for collagenous connective tissue Fibrous connective tissue of endomysial and perimysial can be seen more clearly in m soleus Magnification. Bar = 100 μm

was higher in MS than in MRF but there was no significant difference between the muscles in the hexosamine/hydroxyproline ratio There were no significant differences between the trained and con trol rats in respect of the above concentration values

Table 4 shows the corresponding results on the middle sections of the muscles i e the sections including the actual muscle connective tissue There were significantly more hexosamines in the middle section of MS compared to that of MRF In the hydroxyproline concentration there was how ever a reversed difference between the two mus cles of the untrained animals There was no dif ference between the muscles in the hydroxyproline/ nitrogen ratio but the hexosamine/hydroxyproline ratio was higher for MS compared to MRF percentage of soluble collagen was higher in [illegible] than in MS

If the different concentration values of the [illegible] section of the muscles are compared to th[illegible] whole muscles (cf Tables 3 and 4) lower [illegible] for hydroxyproline and hexosamines can be [illegible]served in the former muscle samples. In fact [illegible] were highly significant differences ($P<0$[illegible] tween the middle and proximal or distal sec[illegible] both muscles in the concentrations of h[illegible] proline and hexosamines The ratio of hexo[illegible] to hydroxyproline was highest in the mid[illegible] tion of the muscles

The PH activities in the two muscles of [illegible] trained and control rats appear in Fig [illegible]

Table 3 *Biochemical properties of connective tissue of m rectus femoris and m soleus (whole mus[illegible] trained and control rats*

Mean ± S D and number of observations are given

Variable	Rats	MRF	MS	P
Hydroxyproline (μg mg d w)	Trained	9 9±1 0 (9)	14 2±1 6 (9)	<0 001
	Control	9 8±0 7 (10)	14 1±1 6 (10)	<0 001
Nitrogen (μg mg d w)	Trained	169±18 (9)	153±1 (9)	<0 05
	Control	171±21 (10)	155±14 (10)	n s
Hexosamine (μg mg d w)	Trained	0 87±0 14 (9)	1 [illegible]5±0 17 (9)	<0 001
	Control	0.86±0 13 (10)	1.20±0 09 (10)	<0 001
Hydroxyproline/nitrogen	Trained	0 053±0 0[illegible]0 (9)	0 093±0 014 (9)	<0 001
	Control	0 059±0 008 (10)	0 092±0.011 (10)	<0 001
Hexosamine/hydroxyproline	Trained	0 087±0 015 (9)	0 089±0 014 (9)	n s
	Control	0 087±0 015 (10)	0 086±0 012 (10)	n s.

Note There were no significant differences between trained and control rats in any o[illegible] les

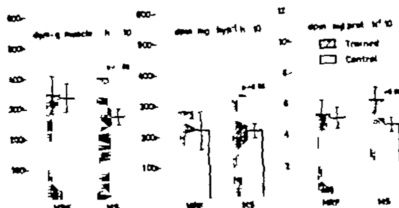


Fig. 2. Prolyl hydroxylase activity of m. rectus femoris and m. soleus in trained and control rats. Mean ± S.E. of 9–10 observations are given.

no significant differences between MRF and The PH activity was however significantly sed by training in MS regardless of the vari- e activities were referred to.

USSION

he lower body weights and the higher heart ts for the trained compared to control animals te that the training was effective enough to about the well-known physiological adapta- to endurance training. As could be expected e basis of previous results (see Holloszy 1975), the endurance training used in this study did not, however induce any hypertrophic growth of the two skeletal muscles.

The decreased LDH activity in MRF of the trained rats is in agreement with previous studies which show that in the muscles with high glycolytic activity the level of glycolytic enzymes decreases in response to endurance exercise (see Holloszy & Booth 1976). The MDH activity has been found to increase up to 50 to 60% following endurance training (Holloszy & Booth 1976). The training effect found in the MDH activity in the present study (10 to 15%) was not, however statis-

4. *Biochemical properties of connective tissue of m. rectus femoris and m. soleus (middle section) ned and control rats*

± S.D. and number of observations are given

le	Rats	MRF		MS		P
hydroxyproline (µg mg d.w.⁻¹)	Trained	8.1±1.4	(9)	7.4±1.3	(9)	n.s.
	Control	7.4±0.4	(10)	6.5±1.0	(10)	<0.05
in (µg mg d.w.⁻¹)	Trained	179+23	(9)	159±16	(9)	n.s.
	Control	166±17	(10)	157±15	(10)	n.s.
(µg mg d.w.⁻¹)	Trained	0.84±0.15	(9)	1.14±0.17	(9)	<0.01
	Control	0.79±0.12	(10)	1.06±0.14	(10)	<0.001
hydroxyproline/nitrogen	Trained	0.046±0.009	(9)	0.047±0.011	(9)	n.s.
	Control	0.046±0.005	(10)	0.042±0.008	(10)	
hydroxyproline	Trained	0.108±0.027	(9)	0.159±0.036	(9)	<0.01
	Control	0.106+0.016	(10)	0.166±0.029	(10)	<0.001
le collagen (%)	Trained	4.29±0.55	(6)	2.38±0.55	(6)	<0.001
	Control	4.25±0.97	(6)	2.95±0.18	(6)	<0.05
soluble protein (%)	Trained	30.5±7.9	(6)	23.8±6.6	(6)	.s
	Control	33.3±7.9	(6)	27.7±.9	(6)	n.s.

There were no significant differences between trained and control rats in any of the variables.

tically significant The LDH activity was three times higher in MRF than in MS whereas the MDH activity was 30 to 40% higher in MS than in MRF This is in agreement with the earlier findings of Holloszy & Booth (1976) which showed that in the fast twitch white and red muscle fibers the activities of glycolytic enzymes are higher than in the slow twitch red fibers in which the activities of aerobic enzymes like MDH are higher On the basis of the fiber composition the two muscles can be considered as clearly different as to their "fast and slow" contractile properties

The collagen concentration was 40 to 50% higher in MS than in MRF when whole muscles were taken into account The very recent findings of Laurent et al (1978) also showed that there is considerably more collagen in the slow anterior latissimus dorsi compared to the fast" posterior latissimus dorsi in the fowl The high collagen concentrations of the tendinous distal and proximal sections of MS at least partly explain the difference between the two muscles in the present study because similar results were not observed in the middle sections of the muscles The histochemical analyses however suggested that there may also be more internal collagen of endomysium and perimysium for MS as against MRF The individual muscle fibers in MS were more distinct than those in MRF when stained according to the Herovici method One reason for the observed differences between MS and MRF may be that since the ST fibers have a richer capillary supply than FT fibers (Andersen & Kroese 1978) the "slow MS probably also has more collagen around the capillaries than the "fast" MRF The relatively high hydroxyproline concentration as well as the low ratio of hexosamines to hydroxyproline in the middle section of MRF is apparently due to a distinct collagenous connective tissue septum which can also be macroscopically observed and which divides the muscle into two parts

There was more collagen in soluble form (i e newly synthetized collagen) in MRF than in MS It can be associated with the "slow" MS is functioning as a static position-maintaining muscle while the "fast" MRF is a more dynamic and active muscle According to Kruggel & Field (1974) the solubility of collagen in an active muscle is higher than in a quiescent muscle The endurance training did not affect the solubility of collagen in either of the muscles in the present study This is in agreement with the studies performed by Suominen [illegible] (1978) which show that even "life-long [illegible] training does not change the solubility of [illegible] collagen when continually training men [illegible] pared to sedentary men.

Hexosamine concentrations were about [illegible] higher in MS than those in MRF when [illegible] muscles were taken into account. The [illegible] concentration of the middle section of MS was [illegible] higher than that of MRF This suggests that [illegible] more ground substance especially glycosamin[illegible] cans in the connective tissue of MS This [illegible] tion is supported by the fact that the ratio of [illegible] amines to hydroxyproline was also higher [illegible] connective tissue of MS However [illegible] with Boas (1953) assumptions, we have [illegible] our later experiments that the Boas method [illegible] excessive values for skeletal muscle [illegible] Consequently the quantitative level of the [illegible] of this experiment is uncertain, but this does [illegible] influence the comparability of the results [illegible] present study

The training program used in this study did [illegible] duce any significant changes in the amount of [illegible] cle connective tissue It has also been observed [illegible] moderate endurance training does not [illegible] increase the concentration of hydroxyproline [illegible] hexosamines in long bones and Achilles [illegible] (Kuskinen 1976) This histochemical analyse[illegible] Müller (1974) indicated a local increase of [illegible] tive tissue in MS but this was obviously [illegible] by overload during the first weeks of training [illegible] plausible that moderate training does not [illegible] change the concentrations of muscle conn[illegible] tissue components

The effect of training on muscle PH activity [illegible] similar to that of MDH activity i e the [illegible] was higher in MS than in MRF This increase [illegible] independent of whether the enzyme activity [illegible] referred to the weight of the muscle sample [illegible] concentration of hydroxyproline or protein of [illegible] muscle sample It can be assumed that the [illegible] creased PH activity of MS reflects the accel[illegible] turnover of collagen This conception is sup[illegible] by our later H-proline incorporation experi[illegible] (Kovanen et al unpublished data) which [illegible] activated collagen metabolism after training [illegible] cording to Suominen & Heikkinen (19[illegible]) Suominen et al (1977) endurance training [illegible] creases the PH activity of human skeletal mus[illegible]

It can be concluded fr[illegible] bservations of

…that endurance training does not change the …cal concentrations of collagen and glycos…glycans in the connective tissue of skeletal …e. The metabolism of collagen may however … celerated. Furthermore it seems that the …en of "fast" and "slow" muscles is not equal… tributed in the different muscle connective … components and that there are at least more …saminoglycans in "slow" than in "fast" …e. More specific analyses on connective … at the level of fibers in different skeletal …les are being performed.

…study was supported by grants from the Finnish …rch Council for Physical Education and Sport …try of Education) and the Academy of Finland. …uthors wish to thank Prof. Antti U. Arstila, Depart… of Cell Biology, University of Jyväskylä and Dr …komäki, Laboratory of Pathology, Central Hos… Jyväskylä for their advice and placing laboratory …ies for the histochemical analyses at our disposal. … also grateful to Mrs Salme Moilanen, Mrs Bertta … and Mr Erkki Helkala for their skillful technical …ance.

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sodilatation in the dental pulp produced by electrical mulation of the inferior alveolar nerve in the cat

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GAZELIUS, B & OLGART L. Vasodilatation in the dental pulp produced by electrical stimulation of the inferior alveolar nerve in the cat. Acta Physiol Scand 1980, 108, 181–186 Received 23 May 1979 ISSN 0001-6772. Department of Pharmacology and Department of Endodontics, Karolinska Institutet, Stockholm, Sweden.

The effects of nerve stimulation on blood flow were studied in the dental pulp of anesthetized cats. Changes in iodide disappearance rate (k-value) from dentinal cavities were used to determine changes in pulpal blood flow Electrical stimulation of the distal end of the cut inferior alveolar nerve after α-adrenoceptor blockade (phentolamine 3 mg/kg) consistently resulted in rapid increase in disappearance rate The first stimulation produced the greatest response (an average increase in k values of 60%) and repeated stimulations showed successive attenuation in response the fourth stimulation usually having no effect. A progressive decrease in resting k-values was observed after the first stimulation, indicating an impaired exchange function of the capillary vessels. Systemic pretreatment with propranolol (0.5–1 mg/kg) atropine (3 mg/kg) mepyramine (3 mg/kg) and cimetidine (3 mg/kg) did not influence the rapid increase in k values produced by the nerve stimulation The experiments show that vasodilatation in the cat dental pulp produced by stimulation of the inferior alveolar nerve is not mediated by common efferent vasodilatory mechanisms and strengthen the hypothesis that the sensory nerve axon reflex mechanism is involved.

Key words Dental pulp, iodide disappearance vasodilatation, sensory nerves

ctrical stimulation of the distal stump of the sec ed mandibular nerve in dogs has been shown to rease intrapulpal tissue pressure (Kroeger 1968) d to increase pulpal blood flow (Tonder & Naess 78) These findings suggest that there is neural sodilatory mechanism in the dental pulp and raise question of whether such neurons are functiony efferent or afferent. The presence of cholinesrase in the pulp may lend support to the concept an efferent cholinergic innervation of the pulp ohto & Antila 1968 Avery et al 1974) It has also en suggested that histamine participates in the somotor regulation in this tissue (Pohto & Antila 78, Edwall et al. 1973). Vasodilatation may also mediated by sensory nerve fibres Antidromic mulation of sensory fibres has long been known induce vasodilatation in the skin in different ecies (Bayliss 1901 Dale & Gaddum 1930 Celan- & Folkow 1953 b). This effect, which is resistant and probably mediated via pain fibres (Celander & Folkow 1953*a*) seems to be related to the axonal reflex mechanism. Such mechanism may con... bute to vasodilatation also in the dental pulp The present investigation was designed to study whether electrical stimulation of the inferior alveolar nerve in the cat can influence pulpal circulation and if possible to establish the nature of the dilatory fibres involved

METHODS

The experiments were carried out on cats (2–4 kg and 1–2 years old), anesthetized with chloralose (40 mg/kg) and urethane (50 mg/kg) The trachea was cannulated and the blood pressure in the femoral artery was recorded Body temperature was kept constant at 38°C by heating lamps.

The inferior alveolar nerve was exposed over length of about 3 cm by removing bone from the mandibular base. The distal stump of the sectioned nerve was stimulated via bipolar silver electrodes at 10–20 Hz 5 ms and 10 V for periods of –4 min, using Grass stimulator A resting period of 10–15 min was allowed between stimulations. In

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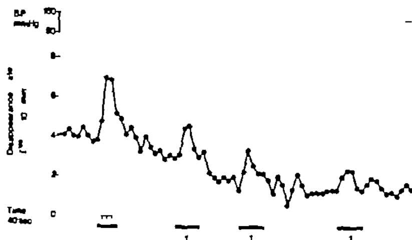


Fig. 1. Influence of inferior alveolar nerve stimulation on pulpal microcirculation. Cat, young adult. Chloralose-urethane. 1. Stimulation with 10 V, 20 Hz, 5 ms. Each k value represents the running average for 40 period.

third stimulation. Similar results were obtained [...]h atropine (3 mg/kg) and mepyramine (3 mg/kg) [...]ther with cimetidine (3 mg/kg) were adminis[...]d in an analogous way. In all these experiments [...] second response was still pronounced and ap[...]ntly unaffected by the drugs since there was no [...]ificant change in the mean difference between [...] first and second response as compared to con[...] (Table 1). In another series of experiments (5 [...]mals) the nerve was stimulated after pretreat[...]nt with all the agents. In this group of animals [...]h the first and the second response appeared unaffected after the drug administration as compared to the control group. Thus, in the experimental group the mean response during the first and second stimulations (1.41±0.23 and 1.11±0.39) did not differ significantly from the corresponding responses in the control group (1.68±0.40 and 1.01±0.27).

Cutting the inferior alveolar nerve

In 5 cats the nerve remained intact within the mandibular canal until it was cut during the recording of pulpal blood flow. In one of these experiments such

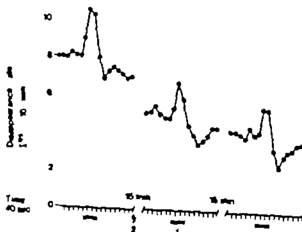


Fig. 2. Influence of inferior alveolar nerve stimulation on pulpal microcirculation before and after systemic administration of propranolol. Cat, young adult. Chloralose-urethane. 1. Stimulation with 10 V, 15 Hz, 5 ms. 2. Propranolol 0.5 mg/kg.

Table I *Increase in k values reflecting increased pulpal blood flow following inferior alveolar nerve stimulation*

All animals were pretreated with phentolamine (3 mg/kg) and the animals in groups A B and C were given the drugs between the first and second stimulations The results are expressed as mean ± S E. and n is the number of teeth

Drug	Stimulation 1st	2nd	Mean difference 1 2
Control phentolamine n=9	1 68±0 40	1 01±0.27	0 67±0 32
A propranolol n=4 (0.5 mg/kg)	2 42±0 49	1 73±0 16	0 69±0 33
B atropine n=4 (3 mg/kg)	1.35±0 44	0 61±0 03	0.74±0 44
C mepyramine cimetidine n=4 (3 mg/kg)	1.35±0.34	0.98±0 02	0.37±0 37

some experiments the cervical sympathetic trunk was exposed cut and stimulated at 3–6 Hz 1 ms and 6 V for 5–10 min.

A cavity was prepared within the gingival half of the crown in the ipsilateral lower canine tooth The cavity was deepened by a hand held end cutting burr until the shadow of the pulp was barely visible through a thin layer of dentin This procedure has previously been reported to cause a minimum of injury to the pulp tissue (Ahlberg & Edwall 1977) Circulatory changes in the pulp were determined by the disappearance technique using a radioactive tracer solution (^{125}I 80 µCi/µl as iodide dissolved in phosphate buffer with sodium thiosulphate) applied as a depot in the tooth cavity A thin plastic film and Plastibase insulating gel were used to cover the cavity to prevent evaporation. Radioactivity was monitored by an external scintillation detector and counted for 40 s periods The technical details of this method have previously been described by Bolme & Edwall (1970) and Edwall & Kindlova (1971) Counting was started 5–10 min after the isotope application when the disappearance rate was found to be monoexponential The disappearance rates were calculated as k values and changes in this parameter were related to changes in pulpal microcirculation (Edwall 1971 Edwall & Kindlova 1971) Increases in k values are expressed as the difference between peak values and the mean of 6 prestimulatory values

Phentolamine (3 mg/kg i v) was given before the experiment to prevent vasoconstrictor effects This dose was previously tested in four animals during stimulation of the cervical sympathetic nerve and was found to abolish the vasoconstrictor response completely after which no increase in disappearance rate occurred An additional dose of 0.5–1 mg/kg was given every hour to ensure a complete α-adrenoceptor blockade throughout the experiment

The changes in k values observed as a result of inferior alveolar nerve stimulation were compared within the same animal before and after (15–20 min) intravenous administration of propranolol (0.5 mg/kg) atropine (3 mg/kg) mepyramine (3 mg/kg) and cimetidine (3 mg/kg) given separately Similar comparisons were made between animals which had received all these drugs and untreated controls Results are expressed as mean ±S E

RESULTS

Inferior alveolar nerve stimulation

Efferent stimulation of the distal stump of the nerve after appropriate α-adrenoceptor blockade caused a mean increase in the k values in the ipsilateral teeth of 1 86±0 46 (29 teeth) In experiments with repeated stimulation (9 teeth) the first stimulation produced a greater increase (mean 1 68±0 40) than the second (mean 1 01±0.27) (Table I). The responses were further attenuated in experiments where successive stimulations were carried out (Fig 1) so that the fourth stimulation usually had little or no effect These results also illustrate the frequent observation that the basal k values decrease progressively during the course of the experiments This finding differs from previous results showing that the disappearance rate from the depot usually is monoexponential under resting conditions for at least one hour after the application of the depot (Edwall & Scott 1971 Olgart & Gazelius 1977) A possible explanation for this continuous drop in the k values is that the exchange function of the capillaries is impaired due to an increased permeability and protein extravasation

Attempts to block the vascular response

The nature of the vasodilator mechanism was explored by pharmacological means Thus, blocking agents were introduced by the systemic route between the first and second stimulations in one series of experiments (16 teeth) The results of one of these experiments in which propranolol (0.5 mg/kg) was given 20 min before nerve stimulation are shown in Fig 2 As can be seen there was still a pronounced increase in k val during the

be explained by such a vascular reaction. An increase in vascular permeability and protein exudation would impair the capillary exchange of solutes from the tissue and thus reduce the rate of clearance of the tracer from the cavity-tissue area. Although this late aspect of the vascular response has not been further analyzed in this study, it also gives some support for the concept of an axon reflex mechanism in the dental pulp.

The identity of the vasoactive substance contained in pain fibres is not known. Our findings that the vasodilatory responses were attenuated following repeated stimulation may indicate that they are dependent on the release of an active neural substance which can be depleted by prolonged electrical stimulation of the nerve. This assumption is interesting and may relate to our previous observation that Substance P-like immunoreactivity (SPLI) is contained within fine calibre nerve fibres in cat dental pulp (Olgart et al. 1977) and that increased amounts of SPLI can be found in superfusates from the exposed cat pulp following nerve stimulations similar to those used in the present study (Olgart et al. 1977*b*). This release was also attenuated following repeated stimulations and very little SPLI was found after the second stimulation. We have also demonstrated that synthetic SP has a powerful vasodilatory action in the cat pulp (Gazelius et al. 1977). Radioimmunoassay of SP in cat pulps revealed amounts of up to 43 ng/g tissue (to be published). The finding of such high amounts of SPLI is not surprising since the pulp is richly innervated and recent histological findings in cats indicate that a large proportion of the pulpal nerves belongs to the group of unmyelinated fibres (Nilsen & Karlsson 1974). The role of these fibres in dental pain has not yet been established but it is possible that vascular reactions in the pulp associated with pain may have a nervous component in which such fine calibre SP-containing nerve fibres are involved.

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a procedure caused a slight and transient increase in k values whereas in the other four cats there was no effect

DISCUSSION

The present experiments in cats confirm and extend the previous observation in dogs (Tönder & Naess 1978) that electrical stimulation of the mandibular nerve causes increased blood flow in the dental pulp In addition we have shown that this effect is not mediated by noradrenergic or cholinergic nerves Our findings that propranolol did not influ ence the vascular response to inferior alveolar nerve stimulation and that phentolamine failed to reverse the constrictor response to sympathetic stimulation are in agreement with previous observations in dogs and cats showing that there is little evidence that there are β-adrenoceptors in the dental pulp (Edwall 1971 Tönder 1976)

It is not clear if there are cholinergic vasodilator fibres in the dental pulp Functional studies in dogs (Weiss et al 1977) and the histochemical demon stration of specific cholinesterase activity related to nerve fibres in the rabbit cat and dog pulp (Avery et al 1971 1974 Pohto & Antila 1972) lend some support to the hypothesis that there is an autonomic vasodilator mechanism in the pulp The results of the present experiments with atropine show how ever that acetylcholine is not involved in vasodila tation produced by inferior alveolar nerve stimula tion and they do not support the idea of a role for cholinergic vasomotor fibres in the cat dental pulp

Histamine-like fluorescence has been found in human cat and rabbit pulps (Pohto & Antil 1972) and mast cell histamine has been suggested to play a part in vasodilatation following electrical stimula tion of cutaneous nerves in rats (Kiernan 1975) Since however in the present study the vascular response was not influenced by mepyramine or cimetidine it is unlikely that histamine is involved

It is thus evident that stimulation of the nerve supply to the dental pulp does not activate common efferent vasodilatory mechanisms and this supports the hypothesis that sensory nerves are involved Sensory nerves have long been known to mediate vascular reactions in the skin of different species For example Celander & Folkow (1953*a* *b*) dem onstrated in the cat that pain fibres in the skin are involved in vasodilatation induced by local noxious stimulation and by antidromic stimulation of a cut cutaneous nerve or dorsal root fibres. It [illegible] that the wellknown axonal reflex mechan[illegible] sociated with unmyelinated pain fibres is [illegible] also in the present experiments. The [illegible] intensity used in our study is within the [illegible] known to stimulate afferent C-fibres in [illegible] dilator responses (Hinsey & Gasser 1930, [illegible] Ladd 1976)

In the present experiments the vasodila[illegible] sponse has been expressed in terms of [illegible] disappearance rate (k value) of iodide (KI) [illegible] According to previous studies changes in [illegible] pearance rate are mainly related to changes in [illegible] pal capillary blood flow (Edwall 1971). There [illegible] however other factors that may influence [illegible] especially at higher flow rates (Angard & [illegible] 1974) For example diffusion barriers be[illegible] dentin and capillary blood would tend to [illegible] crease in k values Such a phenomenon [illegible] explain the slight decrease in k values [illegible] during the last part of the stimulation period [illegible] more pronounced and transient drop below [illegible] values of this parameter after the dilatation [illegible] 2) Furthermore it is possible that during the [illegible] lation a concomitant vasodilation in neighb[illegible] tissues may reduce the effects on pulpal blood [illegible] due to a drop in pulpal perfusion pressure (T[illegible] 1976) Accordingly the vasodilator response [illegible] pulp may have been underestimated in the pre[illegible] study

In addition to vasodilatation antidromic st[illegible] tion of saphenous and trigeminal nerves in [illegible] been shown to cause increased vascular perme[illegible] ty (Jancso Jancso-Gabor & Szolcsanyi 1967 [illegible] Chahl & Ladd 1976) This effect usually oc[illegible] some minutes after the commencement of [illegible] stimulation and has been related to a number [illegible] inflammatory mediators For example plasma [illegible] nins have been implicated in neurogenic ede[illegible] (Kiernan 1975 Arvier et al 1977) and a brad[illegible] like substance has been found in superfusates [illegible] dental pulps of dogs after electrical stimulation [illegible] the pulp or the mandibular nerve (Kroeger [illegible] Inoki et al 1973 1979) It is therefore likely [illegible] antidromic vasodilatation in the pulp is also [illegible] lowed by an increase in vascular permeability T[illegible] phenomenon may explain the sustained increase [illegible] intrapulpal pressure following antidromic [illegible] stimulation as reported by Kroeger (1968) [illegible] progressive decrease in k values seen in our st[illegible] after commencement of [illegible] stimulation [illegible]

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e kallikrein and kinin excretions in dogs during l artery constriction and release

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kallikrein originates intrarenally (Nustad Orlwaki et al 1976), and the preenzyme is in distal tubular cells (Orstavik et al. 1976) elease into the tubular fluid kallikrein might potent and active peptides, kinines from e (kininogen) present in preurine Thus, rmation at a distal tubular level is supported flow analyses (Scicli et al 1978).

e past urine kallikrein excretion has been s an index of "intrarenal kinin activity reports, however show that urine kallikrein e kinin excretions might vary independent ci et al. 1978, Olsen 1978) and it has been ted that substrate availability rather than e activity might be the rate limiting step in atubular kinin forming process.

e kallikrein is decreased during partial renal constriction (Mills et al 1976, Keiser et al and a transient enhancement of enzyme ex has been noted following cessation of the ction (Mills et al. 1976) Consequently the study was initiated on the idea that acute porary changes of renal blood flow might be ment model to study the interrelationships en urine kallikrein and urine kinin excretions ms shed additional light on the possible phy- cal significance of the peptides

female mongrel dogs (13–23 kg) which had fasted overnight were used for the experi-

The dogs were anesthetized with pento- al sodium and surgically prepared with a sling for manual constriction placed around eft renal artery distal to an electromagnetic meter probe (Olsen 1978). Following comple- f surgery the dogs were loaded with 100 ml mannitol in saline (10 ml/min) and 2 ml/min nfused throughout the experiment When urine had stabilized about 45 min after surgery ntrol urine was collected for 15 min. Left renal l flow then reduced in tw steps In the first slight reduction (to approximately 90 % of control) ensured maximal autoregulatory vaso- dilation. In the second step renal blood flow was markedly reduced to approximately 50 % of control. At each step the initial 1 ml urine was discarded (in the ureteral cannula dead space volume was 200 µl) and thereafter urine was collected for 15 min After release of the constriction the experiments were concluded by three 15 min postcontrol urine collec tions. Urine kallikrein was determined as (1) TAMe-esterase activity (Margolius et al 1974 Olsen et al. 1979) and (2) kininogenase activity (Olsen 1978) Subsequent to extraction kinins were assayed biologically (Olsen 1978) and sodium was determined by flame photometry

The present study shows (Table 1) that the rate of urine kinin excretion decreased by renal artery con- striction. The fall of peptide excretion was related to the degree of renal blood flow reduction and was in parallel with decreased urine kallikrein excretion. Upon release of the constriction renal blood flow rapidly normalized This was followed by a tempo- rary enhancement of kallikrein excretion, whilst urine kinin excretion remained reduced or in fact in 5 of the 10 experiments was temporarily de creased to a level lower than that achieved during the blood flow reductions This shows that urine kallikrein and urine kinin excretions may vary in- dependently Apparently enzyme activity is not a major determinant of the intratubular kinin forma- tion A similar conclusion has been drawn from experiments where urine kinin excretion was in- creased in association with unchanged or decreased kallikrein as observed in dogs during renal venous constriction (Olsen 1978) or indomethacin treat ment to patients with Bartters syndrome (Vinci et al 1978) Consequently the results suggest that substrate accessibility might be the limiting step in the intratubular kinin forming process. As a corol lary this would imply that renal artery constriction might hav modified the mechanism(s) by which kininogen becomes available to enzyme Th hypo-

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te on periodic sweating

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the character of sweat discharge from the sweat glands has been described in several s (Randall 1946 Rothman 1954 Bullard 1962, e 196.., van Beaumont 1969 Kuypers & 197?). Various periods, ranging from one down to a few sec have been observed.

periods of a few sec are most obvious in gl sweating and are probably the expression ation of the autonomic nervous system k Palmes 1951–52, Cotton et al. 1975)

ndes & Sevadjian (197?) found short-period ns) pulsatile expulsions of sweat by each and. They claimed that these expulsions are contractions of the myoepithelial cells. The of myoepithelial cell origin explaining the ions is supported by Nakayama & Takagi ho reported periods of 3–9 s.

n et al (1975) used Fourier analysis to ne individual frequencies in sweating.

ntly Bini et al (1979) showed (by microelec recordings from cutaneous nerve fascicles) domotor impulses appear in bursts occurring t intervals, which are integer multiples of 0 6 s. These rhythmically occurring sudo- nerve responses were accompanied by elec nal responses The results of Bini et al support the theory that the sweat periodicity rned by the sympathetic nervous system

aim of this work was to study further the icity of evaporation prior to and during of thermal eating, using a fast responding ment for continuous measurement (N lsson

measurement principle based on the esti n of the apour pressure gradient of the air close to the skin I the vicinity of the evapo- surface the following equation is valid

$$D'\frac{\delta p}{\delta x}$$

re

is the amount of water evaporated per unit time and area (g/m² h) in this paper ex pressed as evaporation rate

D' is a constant (0 670×10⁻ g/mhPa)

$\frac{\delta p}{\delta x}$ is the vapour pressure gradient (Pa/m)

The evaporation rate is proportional to the vapour pressure difference of two points situated on a line perpendicular to the evaporative surface The vapour pressure at each point is calculated as the product of the relative humidity and the saturated vapour pressure the latter a function of the temperature alone. The response time of the instrument, which is limited by the time constants of the temperature and humidity sensors, is short (0.3 s at 25°C) In addition the measurement method influences the evaporation process only to a minimal extent.

The material consisted of 18 healthy subjects 13 men and 5 women, ranging in age from 26 to 37 years. The measurements were performed at an ambient temperature of 24.8±2.5°C (mean ± S D) and a relative humidity of 34±10% (mean ± S.D)

The experiment was initiated by letting the subjects sit a few minutes at rest on a cycle ergometer They then started to exercise on the cycle ergometer (mean work load 65 W) for 10 min, during which time period sweating broke out within a few minutes. During the entire experiment the evaporation rates from the flexor side of the right forearm, were continuously measured by the evaporimeter The evaporation rates were recorded on an instrumentation tape recorder

Time intervals of the duration of 1 min were selected. Analysis of the selected passages was performed by autocorrelation (maximum time delay τ=5 s) in a mini-computer

The autocorrelation functions (ACFs) were calculated from the values of the evaporation rates during the exercise period. In all 18 subjects a similar pattern of the ACF was found during the time period prior to active weating as recorded by the evaporimeter The average ACF of the 18 subjects is shown in Fig. 1

A periodicity can be observed i the autocorrelation function. The period is 0 74±0.06 s (mean ±

Table 1 *The effect of renal artery constriction (RAC) on kidney parameters*
Results are mean ± S E (N=10) *=$p<0.05$ **=$p<0.01$ by Wilcoxon matched pairs signed ranks test

	Precontrol	RAC-step 1	RAC-step 2	Postcontrol		
	0–15 min	15–30 min	30–45 min	45–60 min	60–75 min	75–[illegible]
Renal blood flow ml/min	151±[illegible]2	133±19*	86±13	155±[illegible]	161± 1	[illegible]
Urine flow ml/min	1 6±0	1[illegible]±0 [illegible]	0 8±0[illegible]	1.5±0[illegible]	1 7±0 [illegible]	[illegible]
Sodium excr µmol/min	118±26	76±19*	41±17	86±15	11 [illegible]	[illegible]
TAMe-esterase mEU/min	0 18±0 03	0 17±0 03	0 13±0.04	0.24±0.03	0 18±0 03	[illegible]
Kininogenase U/min	3±0 4	2 0±0 4	1.3±0.3	[illegible]6±0.5	[illegible]3±0.4	[illegible]
Kinin excr ng/min	3 4±0 7	2 6±0.5	2 0±0 4	1 7±0.7	2.6±0 [illegible]	[illegible]

thesis however remains to be evaluated Alternatively though less comprehensible reductions of urine kinin excretion by renal artery constriction might be due to increased intrarenal degradation of kinins In dogs kininase inhibition by SQ 14225 or SQ 20885 has been shown markedly to enhance the appearance of kinins in urine (Nasjletti et al 1975 Olsen & Arrigoni Martelli 1979) thus suggesting that kininase activity might also determine urine peptide excretion

The investigations of the role of intrarenally generated kinins are hampered by the lack of specific kinin inhibitors Infused into the renal artery the peptides are potent vasodilators that produce natriuresis (Jacobson 1970) The present data however do not permit any conclusion about the possible physiological role of kinins formed intrarenally The immediate normalization of blood flow associated with reduced kinin excretion following cessation of renal artery constriction shows that intratubularly generated kinins exhibited minor (if any) hemodynamic function Also supporting this view are our previous experiments in dogs which showed that increased intratubular kinin generation by renal venous constriction apparently did not affect renal blood flow (Olsen 1978)

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ance for a histamine H_2 receptor involvement in ;dine s antihypertensive effects during multiple dosing

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-al α-adrenoceptor agonist action has been ted as the main mechanism of clonidine s ressure lowering effect (Schmitt et al. 1971 t 1977) That concept has been questioned involvement of central histamine H_2-recep- s been suggested by Karppanen et al (1976 Most of the cited studies have however been ned in the anesthetized normotensive animal ular injections which allow no conclusions as antihypertensive mechanism in particular multiple dosing During such a circumstance ne (>0 5 ng/ml) has been found to induce a tration dependent blood pressure decrease spontaneous hypertensive rat [(SHR) Chris- n et al. 1979] with maximal blood decrease en plasma concentrations of 1–4 ng/ml At trations above 4 ng/ml the antihypertensive duces. In the present study the influence of dine (a clinically used histamine H_2-antago- as studied on the blood pressure effects of ine at low plasma concentrations—giving a essure response—and at the high concentra- hen the hypotensive effect is reduced.

R of the Okamoto strain were used (mean t 200+10 g) An indwelling carotid arterial ter (vialon o d 0 023) and a jugular vein ter were implanted during barbitone anes- and exteriorized to the back of the neck of t After recovery from the operation (after 1 2 the blood pressure was recorded n the con- n freely moving animal by pressure transduc tatham Pb 23) connected to Grass polygraph dine was of sed at two consecutive rates rding to the technique described by Wagner) and applied by Christensson et al (1979) technique enables steady state concentrations e drug to be reached rapidly Plasma con- rations of clonidine of 1 ng/ml and 10 ng/ml studied At the end of the experiment which d up to 3 h, blood samples for clonidine deter n were taken to ensure that the predicted concentration was obtained Clonidine was assayed by a gas liquid chromatography method (Edlund & Paalzow 1977) as described (Christensson et al 1979) Cimetidine (kindly supplied by Smith Klein & French) 500–1 000 μg was given as a bolus i v 60 min before steady state concentrations of clonidine and during steady state conditions 5–9 expts. were performed at each steady state level. Values mean ± S D are given as % decrease of blood pressure in relation to pretreatment period (mean value of 3 measurements in each rat during 1 h) Significance between observations were obtained by the paired t test

The mean values of the blood pressure in the freely moving rat was 175±10 mmHg. Clonidine at a plasma concentration of 1 ng/ml reduced the blood pressure 7±3 4% at 60 min and 15±3 4% at 180 min after the start of the infusion (Fig 1) No blood pressure decrease was found at 60 min at plasma concentrations of 10 ng/ml but after 180 min a slight decrease of 8±4 3 % was observed Cimetidine alone (500–1 000 μg) did not influence the blood pressure or the heart rate. Pretreatment with cimetidine abolished the hypotensive effect of clonidine (1 ng/ml) (see Figs. 1 and 2) e.g the blood pressure was unchanged or somewhat elevated (6±3%) compared to the pretreatment levels. Cimetidine did not influence the blood pressure effect when the plasma concentration was 10 ng/ml. Bolus injection of cimetidine did not affect clonidine s antihypertensive effect when hypotension was achieved. The plasma concentrations of clonidine (1 and 10 ng/ml) were not altered by cimetidine—the measured values at the end of the infusion was 0 93±0.2 and 11±2.3 ng/ml—indicating that the kinetics of clonidine were not changed by cimetidine

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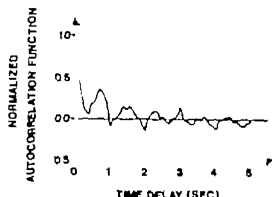


Fig 1 The calculated autocorrelation function (mean of 18 subjects) of the evaporation rate during exercise on a cycle ergometer

S D) A periodical pattern was also found although less pronounced after the onset of sweating The difference between our result and many of the earlier reported periods of the evaporation rate variations probably has its origin in the different measuring techniques used and in different experimental conditions However the result reported in this paper does not exclude the existence of other periods reported by earlier investigators Our result (a sweating period of 0 74 s) is in agreement with the periodicity of around 0 6 s of the sudomotor nerve impulses reported by Bini et al (1979) These similarities suggest that the variations in the evaporation rate directly reflect the periodic activity in these nerves

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Evidence for a histamine H_2-receptor involvement in clonidine's antihypertensive effects during multiple dosing

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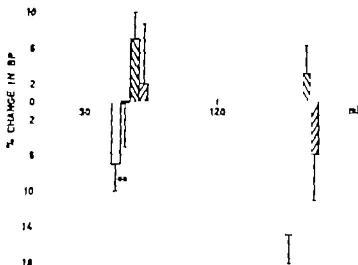


Fig 1 Hypotensive effect of clonidine alone at steady state plasma concentrations (Cpss) of 1 ng/ml (open bars) and 10 ng/ml (black bars) and cimetidine pretreatment 500 µg i v at corresponding steady state concentrations of 1 ng/ml (▧) and 10 ng/ml (▨) Values are mean ± S D ($n=5$–9) denotes significance at the 1% level when compared to the effect after pretreatment with cimetidine

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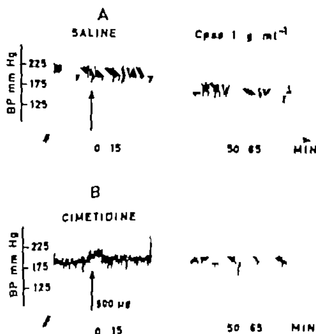


Fig 2 Influence of saline (A) and cimetidine (500 µg) (B) on clonidine s hypotensive effect at steady state concentrations of 1 ng/ml The tracings are from 2 separate rats The pretreatment injection was given 60 min before steady state concentrations were achieved Horizontal axis time course Vertical axis mean arterial pressure mmHg

The skilful technical assistance of Mrs Inger Li[...] greatly acknowledged The investigation [was supported] by the Swedish medical research council B [...] 01

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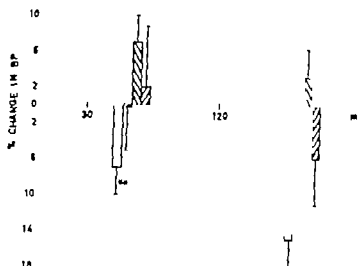


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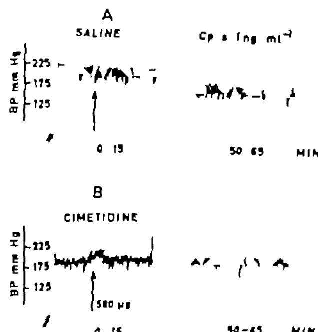


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ne mode of action of botulinum toxin

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num toxin (BoTx) blocks irreversibly and gh specificity the quantal release of acetyl- from motor nerve terminals. The extreme y of the toxin, only a few hundred molecules required to block each synapse (Habig & ra 1979) makes it interesting to speculate its mode of action Taking in account the ation that transmitter release facilitates the g effect of BoTx it has been suggested that on molecule might block the channel or the site from which acetylcholine quanta are ed and that the t xin can only enter into the ng position when there is opening of either a sting closed site or a site created de novo nerve stimulation (Lamanna 1976)

purpose of the present study was to test this hesis. 4-aminopyridine is a drug which, by ating the influx of calcium ions into nerve nals in response to nerve stimuli causes a ve quantal release (several thousands of a) of transmitter (Molgo et al 1977 Lundh As shown by Heuser et al (1979) the number aptic vesicle openings in the nerve terminal ealed by freeze-fracture technique is similar reased by the drug Once BoTx has produced romuscular block 4-AP i a very effective onist (Lundh et al 1977)

ided that the previously mentioned hypo- regarding the mode of action of BoTx was ct, one could expect that if BoTx and 4-AP administered together the toxin should be able ock not only the normally activated release for acetylcholine but also the new sites opened e presence of 4-AP Hence one could expect with a combined administration of BoTx and the number of release sites blocked should be er than ith BoTx alone and as a consequence is 4-AP once the block was established, should ss effective or even ineffective in restoring omuscular transmi sion.

periments were carried out on the xtensor orum longus muscle of male Sprague Dawley rats (160–230 g) The muscle was poisoned by a single subcutaneous injection of BoTx type A in the hind-leg as described by Cull-Candy et al. (1976) The dose of the toxin caused complete paralysis of the leg lasting several weeks. Some of the animals received at the time of poisoning an intraperitoneal injection of 4-AP (3 mg/kg b wt.) or 1 h after BoTx poisoning 2.5 mg/kg 4-AP and 4 h later an additional 1.25 mg/kg of the drug. These doses of 4-AP were the maximal tolerated by the animals, larger doses causing generalized convulsions and death 4 days after BoTx poisoning the extensor digitorum longus muscle with its motor nerve was removed under ether anesthesia from animals given only BoTx and from animals given BoTx+4-AP placed in a constant temperature bath (30°C) and perfused with

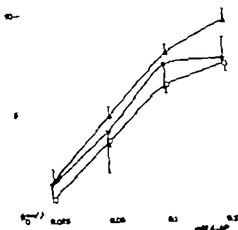


Fig 1 Isometric twitch tension of isolated extensor digitorum longus muscles in response to indirect stimulation at 0.1 Hz. Dose-response curves are given for cumulative increasing concentrations of 4-AP on 9 muscles treated with BoT only (O), 3 muscles treated ith BoT and one 4-AP injection (▲) and 5 muscles given BoT and 2 4-AP injections (▼). There is significant difference ($p<0.05$ Student' test) between the upper and lower curve at the tw highest drug concentrations. Each value is the mean S E

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Carbonic anhydrase in the lung

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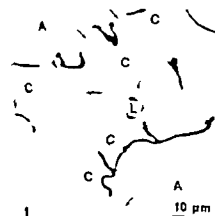
Carbonic anhydrase activity has been demonstrated in lung tissue of human fetus (Berfenstam 1952), rhesus monkey (Fisher 1961) fetal lamb (Ericsson & Waxman 1976) adult rabbit (Effros et al. 1978) and rat (Maren 1967 Crandall & O'Brasky 1978). The localization and function of the enzyme in the lung of these and other mammalian species is unknown, however The aim of the present study was to clarify the distribution of carbonic anhydrase in rat and monkey lung by a histochemical technique.

Lungs of adult male Sprague-Dawley rats and cynomolgus monkeys were perfused at room temperature with saline followed by 2.5% glutaraldehyde buffered to pH 7.4. Small pieces of tissue were then immersed in the fixative for 2 h, embedded in JB-4 plastic, sectioned and stained for light and electron microscopy. Hansson's method for the histochemical demonstration of carbonic anhydrase activity (Hansson 1967) was used in a slightly modified form as described by Ridderstråle (1976). The method and the relevant literature have been discussed by Lönnerholm (1979).

Controls were run with 10 µM acetazolamide added to the incubation medium and this concentration of the inhibitor always abolished visible staining.

In the *rat lung* staining was found only at the structures which separate capillaries and alveoli, i.e. the alveolar-capillary barrier (Fig. 1). In the electron microscope the precipitate was found mainly at the capillary side (Fig. 2 arrows) of this barrier which consists of capillary endothelium, basement membranes and alveolar epithelium (Weibel 1969). The exact localization of the staining is not clear at present, however Those parts of the capillary walls which were not in close contact with alveoli were unstained (Figs. 1 2). Where alveoli were not in close contact with capillaries, their lining structures were unstained (Fig. 1 arrows)

In the *monkey lung* another staining pattern was found. Stain deposits were seen along the whole circumference of the capillaries (Fig. 3). The structures lining the alveoli were unstained at least



1 Rat lung. Stain deposits are confined to the thin tures separating capillaries, C and alveoli, A. Note here alveoli are not in close contact with capillaries ning structures are unstained (arrow). One capillary was an unstained leukocyte L. Incubation time 5 Light microscopy Phase contrast

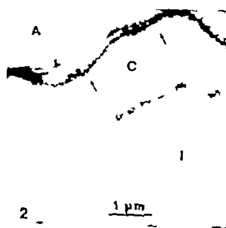


Fig. 2. Rat lung. The stain is located mainly at the capillary side (arrows) of the structures separating capillary C and alveolus A. The capillary wall on the contrary side, which is in contact with interstitial tissue I is unstained. Incubation time 6 min. Electron microscopy

oxygenated medium of the following composition in mM NaCl 135 $NaHCO_3$ 15 0 Na_2HPO_4 1 0 KCl 5 0 $CaCl_2$ 2 0 $MgCl_2$ 1 0 glucose 11 0 The pH of the solution was 7 2–7.3

Isometric twitch tensions were recorded by of a Grass FT-03 transducer connected to an ink writing oscillograph The resting tension of the muscle was adjusted to give maximal twitch response The nerve was stimulated by a glass capillary suction electrode with supramaximal voltage and pulses of 0 05 ms duration at 0 1 Hz Cumulative dose-response curves for the effectiveness of 4 AP to restore neuromuscular transmission were con structed allowing 10 min between each increase in drug concentration

As shown by Fig 1 4-AP was somewhat more effective in restoring neuromuscular transmission in those preparations which 4 days previously had received both BoTx and 4 AP as compared to BoTx only Thus there is no indication that BoTx pre ferentially blocks activated acetylcholine release sites in the nerve terminal membrane It appears necessary to consider some other mechanism of action of the toxin on the probability of transmitter release possibly one reflecting the calcium require ment of the release process as suggested by Cull Candy et al (1976)

The study was supported by grants from the S[illegible] Medical Research Council, Stockholm, Swe[illegible] 311) the Muscular Dystrophy Association of [illegible] Inc and from the Medical Faculty Lund Univ Lund Sweden

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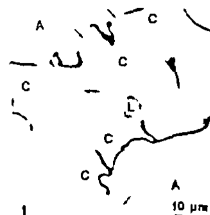
…ic anhydrase activity has been demonstrated …t tissue of human fetus (Berfenstam 1952) …hesus monkey (Fisher 1961) fetal lamb …rson & Waxman 1976), adult rabbit (Effros et …78) and rat (Maren 1967 Crandall & O'Brasky … The localization and function of the enzyme … lung of these and other mammalian species …nown, however The aim of the present study … clarify the distribution of carbonic anhydrase … rat and monkey lung by a histochemical tech-

…gs of adult male Sprague-Dawley rats and cyno… monkeys were perfused … with saline fol… 2.5% glutaraldehyde buffered to pH 7.4 Small … of tissue were then immersed in the fixative for 2 …edded in JB-4 plastic, sectioned and stained for …d electron microscopy Hansson method for the …emical demonstration of carbonic anhydrase activ…sson 1967) was used in slightly modified form …sed by Ridderstråle (1976) The method and the …t location have been discussed by Lönnerholm …975)

Controls were run with 10 μM acetazolamide added to the incubation medium and this concentration of the inhibitor always abolished visible staining

In the *rat lung* staining was found only at the structures which separate capillaries and alveoli, i.e the alveolar-capillary barrier (Fig. 1). In the electron microscope the precipitate was found mainly at the capillary side (Fig 2 arrows) of this barrier which consists of capillary endothelium, basement membranes, and alveolar epithelium (Weibel 1969) The exact localization of the staining is not clear at present however Those parts of the capillary walls which were not in close contact with alveoli were unstained (Figs 1 2) Where alveoli were not in close contact with capillaries, their lining structures were unstained (Fig. 1 arrows).

In the *monkey lung* another staining pattern was found. Stain deposits were seen along the whole circumference of the capillaries (Fig. 3) The structures lining the alveoli were unstained, at least



1 Rat lung. Stain deposits are confined to the thin …ures separating capillaries, C and alveoli, A. Note …here alveoli are not in close contact with capillaries, …ing structures are unstained (arrows) One capillary …ains an unstained leukocyte L. Incubation time 5 … Light microscopy Phase contrast

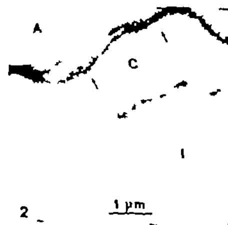


Fig 2 Rat lung. The stain is located mainly at the capillary side (*arrows*) of the structures separating capillary C and alveolus A The capillary wall on the contrary side which is in contact with interstitial tissue I is unstained Incubation time 6 min. Electron microscopy

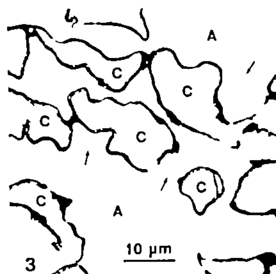


Fig 3 Monkey lung. Stain deposits are present along the whole circumference of the capillaries *C* The lining of the alveoli *A* is unstained at least where they are not in close contact with capillaries (*arrows*) Incubation time 8 min Light microscopy Phase contrast

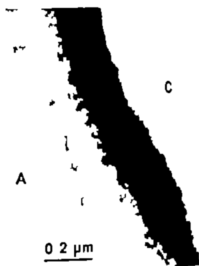


Fig 4 Monkey lung High power magnification of structures separating a capillary *C* and an alveol showing stained capillary endothelium. Incubation t min Electron microscopy

where they were not in close contact with capillaries (Fig. 3 arrows) In the electron microscope the staining was found at the capillary side of the alveolar-capillary barrier (Fig 4) Although the results suggest that carbonic anhydrase is located in the capillary endothelium further work is needed to clarify the ultrastructural localization of the enzyme

A major difficulty in the study of carbonic anhydrase in various tissues is to avoid contamination with enzyme from erythrocytes In the present study the following points argue against the near at-hand suspicion that the observed staining could be due to a fixation artifact and caused by enzyme from ruptured erythrocytes 1) A similar staining pattern was seen in unperfused lungs and extensively perfused lungs where no or very few erythrocytes remained 2) The whole circumference of the rat lung capillaries did not stain which one should expect if the staining was due to enzyme from erythrocytes

The function of carbonic anhydrase in the lung is not clear at present It has been suggested that the enzyme facilitates CO_2 transport through the lung tissue (Enns & Hill 1975) The present demonstration that the enzyme is indeed located at the alveolar-capillary barrier in the rat and monkey lung could agree with a role for it in the exchange of CO_2 over this barrier

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...ulatory effects of decerebration ...e unanesthetized spontaneously ...rtensive rat[1]

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...pertension developed in the spontaneously ...ensive rat (SHR) is dependent on the activity ... sympathetic nervous system (see Yamori ... Thus the brain is of fundamental importance ...ntenance of the sympathetic activity is well ... This is true also for the SHR as indicated ... finding that pithing or ganglionic blockade ...es the blood pressure to the same low level ... SHR as in the normotensive control rat ...rato 1969 Shibayama et al 1971 Iriuchijima ... Albrecht et al 1975) Some studies indicate ... that brain mechanisms could be actively in... [illegible] responsible for) the development of ...ension in the SHR (Haeusler et al. 1972 ...da et al 1976 Nakamura & Nakamura 1977)

... blood pressure and heart frequency of ...e Dawley normotensive unanesthetized rats ...sed markedly after decerebration or a pre ...thalamic transection (Trolin 1975) This would ... to indicate that in the rat brain centers rostral ... lesions normally have a net inhibitory effect ... sympathetic outflow In view of the pro... difference in the central nervous activity in ...HR compared to the normotensive rat, it was ...be of interest to study the circulatory effects ...erebration also in the unanesthetized SHR.

...ods Male SHR of the Okamoto strain, Wistar ... normotensive rats (WK) (Møllegaard, Denmark) ... Sprague Dawley (SD) (Anticimex Sweden) rats ...20 g) were used T... types of brain lesions were ...med. For decerebration at the midcollicular level ...ull was opened immediately caudal to the sutura ...dea and for transection rostral to the hypo... ...mus (prehypothalamic transection) the skull was ...ed on both sides of the sutura coronalis just rostral ... sutura lambdoidea After opening of the skull the ...as sutured and the rat was left to recover from ...eration for ... days On day 3 ... catheter was ins...ed into the left common carotid artery as described ...rolin (1975) On day 4 the basal blood pressure and heart frequency were recorded for at least 30 min. There after the rat was disconnected from the transducer and under a shortlasting halothane (Halothane™ Hoechst) anesthesia the old operation wound of the skull was opened and the decerebration or prehypothalamic transection was performed blindly with a spatula through the pre made opening(s) of the skull (see Trolin 1975). The rat was quickly re-connected to the polygraph and the blood pressure and heart frequency were recorded for one hour The duration of the anesthesia was never more than 3 min. A few non-lesioned rats recovered within a few minutes from this anesthesia At the end of each experiment the level and completeness of the transection was checked Only completely and correctly transected rats were considered for statistical analysis. Student's *t*-test after analysis of variance by two independent criteria of classification or Student' *t*-test alone was performed to calculate significances of differences. P-values less than 5% were regarded as significant.

Results The basal blood pressure before decerebration was significantly higher in the SHR (154 mmHg ±6.3) than in the WK or SD rats (124±7.3 and 120±6.4 respectively). The basal heart frequency was equal in the SHR and SD rat (385 beats/min ±15 and 372±12 resp) but significantly lower in the WK rat (300±3.2).

After decerebration the blood pressure and heart frequency increased significantly in the SD and WK rats. The increase in the SD group showed less variation than in the WK group. However in the SHR the decerebration neither produced increase in blood pressure nor in heart frequency

One hour after decerebration the blood pressures of the 3 groups were not significantly different from each other while the heart frequencies of the SD and WK groups were both significantly higher than the heart frequency of the SHR group (Fig. 1)

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[1] A preliminary report was presented at the congress of S B F.C. 1978, São Paulo Brazil.

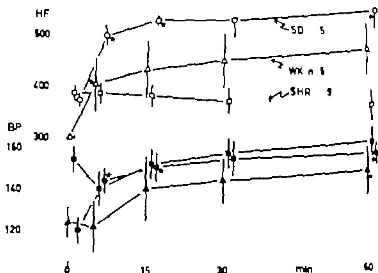


Fig 1 Changes in mean arterial blood pressure (B P mmHg, closed symbols) and heart frequency (H F beats/min open symbols) after decerebration (time 0 min) in Sprague Dawley (SD circles *n*=5) Wistar Kyoto (WK triangles *n*=5) and spontaneously hypertensive (SHR squares *n*=9) rats The rats were anesthetized for -3 min during the decerebration The values are means indicated with S E Values significantly different ($P<0.05$) from the basal value of the same group are indicated with stars

Essentially the same results were obtained after a prehypothalamic transection of the brain (data not shown)

Discussion The pronounced increases in blood pressure and heart frequency after decerebration of unanesthetised SD rats were similar to the effects reported earlier (Trolin 1975) The same results were now also obtained with WK rats The only difference was that in this group the development of hypertension and tachycardia showed more variations than in the SD group On the other hand in the SHR decerebration did not produce any circulatory effects That the pressure did not increase after decerebration in this group could of course be explained by the high initial pressure However neither did the heart frequency increase after decerebration in this group The easiest explanation for this difference between SHR WK and SD rats seems to be that decerebration causes an increase in sympathetic activity in the WK and SD groups but not in the group of SHR It seems safe to conclude that the mechanism responsible for this difference between the strains is to be found within the CNS One possible explanation could be that an inhibitory pathway descending from higher centers (e g Folkow et al 1959) is normally active in normotensive rats but of less importance maybe even lacking, in the SHR

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nal EDL and diaphragm muscles differ eir sensitivity to tetrodotoxin

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nal skeletal muscle the action potential is lly blocked by tetrodotoxin (TTX 10^{-6} M) 966). However after denervation resistance X appears (Redfern & Thesleff 1971) along her striking membrane changes (for a review rves 1976) In a recent work we showed that denervation of the rat extensor digitorum (EDL) muscle nduces TTX resistance not the denervated but also although to a lesser in the endplate region of adjacent active ated fibres (Cangiano & Lutzemberger This and other (Jones & Vrbová 1974 Lømo rgaard 1976, Brown et al 1978) findings te that nerve degeneration induces denerva-ike changes in muscle also through mechan-not depending on muscle inactivity However sults have been questioned by Tiedt et al 4) ho report the presence of TTX resistance endplate region of many normal EDL fibres although in an earlier report (Tiedt et al. b) such fibres are said to be rare (<1%) They emphasize the presence of TTX resistance in endplate region of many normal diaphragm as reported by Thesleff et al. (1974).

order to be able to nterpret the effects of al denervation correctly it is important to blish hether the remaining innervated fibres lop a genuine ncrease in TTX resistance We therefore reexamined the TTX resistance of ally innervated EDL and diaphragm muscles. ormal diaphragm and EDL muscles from male tar rat (280–350 g) were dissected free and ed in a chamber containing 25 ml of an oxy-ated mammalian Ringer's solution at 28°C pH 7 3 containing TTX 10^{-6} M In the endplate ion, identified by the presence of fast rising mini-re endplate potentials, the fibres were penetrat ith closely adjacent conventional recording l current injecting micropipettes. The resting mbrane potential (RMP) was set at −100 and −120 mV by a continuous hyperpolarizing current and action potentials were generated by superim-posed depolarizing current pulses of 12 ms duration. The rate of rise of the action potential was meas-ured simultaneously and its maximal value taken as a measure of the resistance to TTX. This value was influenced by the intensity of the depolarizing pulse strong pulses eliciting action potentials with shorter latencies and faster rates of rise (see an example in Fig. 1B) The pulse was therefore ad-justed to generate action potentials at constant latencies of 4 and 2 ms In this way the EDL and the diaphragm muscles could be examined under strictly comparable conditions in the same bath

As shown in Fig. 1A and Table 1 most of the fibres in the normal diaphragm give action poten-tials at 4 ms in the presence of 10^{-6} M TTX although the rates of rise (and amplitudes) are much reduced compared to normal This is in agreement with Thesleff et al. (1974) In contrast most of the fibres in the EDL fail to give any regenerative response at 4 ms in agreement with our earlier report (Can-giano & Lutzemberger 1977) At a latency of ms

Table 1 *Maximal rate of rise (volt/sec mean ± standard error) of regenerative response elicited in TTX 10^{-6} M from the end-plate region of normal diaphragm and EDL muscle fibres at two different delays (2 and 4 ms) and resting pot ntials set at −100 and −120 mV*

Each group is composed of 32 to 38 fibres from 4 muscles

	2 ms	4 ms
EDL		
100 mV	2.5±1.5	0 0
120 mV	39 1±5.3	5 4_1 9
Diaphragm		
100 mV	51 9±4 4	27 9±3.0
120 mV	75 9 6.2	56 4±4 3

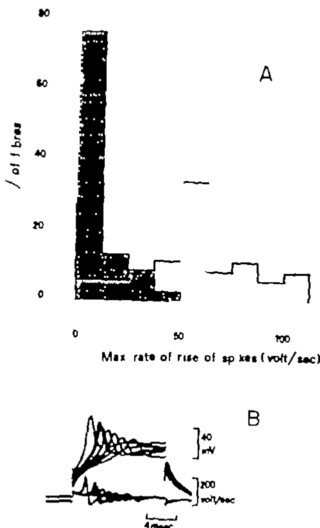


Fig 1 (A) Distribution of resistance to TTX *measured* as the maximal rate of rise of the action potential in normal diaphragm fibres (open columns 36 fibres 4 muscles) and in normal EDL fibres (dashed columns 38 fibres 4 muscles) All measurements *performed at the* neuromuscular junction resting membrane potential set at −120 mV by DC hyperpolarizing current constant delay of 4 ms between onset of the depolarizing pulse and firing of the regenerative response (B) Regenerative responses of a diaphragm fibre in TTX illustrating the dependence of the rate of rise on the delay between onset of the depolarizing pulse and attainment of threshold The first derivative (bottom trace) of membrane potential changes (top trace) was obtained with an RC circuit (100 kohms 100 pFarad) Resting potential set at −100 mV

and a membrane potential of −120 mV however small action potentials appear whose rates of rise are much slower than those elicited in the diaphragm under the same conditions (Table 1)

It is clear from these results that the normal diaphragm is considerably more resistant to TTX in the endplate region than the EDL. The reason for this is unclear It is unlikely to be due to the

presence of a larger number of slow fibres in diaphragm (Korneliussen & Wærhaug 19… cause the slow soleus is affected as strongly a… fast EDL (unpublished observations).

The most important conclusion of this … that under the conditions used in our earlier … (Cangiano & Lutzemberger 1977) when … potentials were elicited at a constant … 4 ms there is essentially no resistance to TTX … the endplate region of normal EDL fibres … the development of TTX resistance in the … denervated muscles previously reported … (Cangiano & Lutzemberger 1977) is a real phe… non indicating that nerve degeneration c… utes with inactivity of the muscle (Lømo & … gaard 1976) to its response to denervation … different conclusion reached by Thesleff et al (19… could arise because they used different … consistent criteria for assessing the TTX resi…

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-trical field stimulation of myometrial strips 1 non pregnant and pregnant guinea pigs

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ELMÉR, M ALM, P & THORBERT G Electrical field stimulation of myometrial strips from non-pregnant and pregnant guinea-pigs Acta Physiol Scand 1980, 108: 209–213 Received 28 5 1979 ISSN 0001-6772 Departments of Physiology Histology Pathology and Obstetrics and Gynecology University of Lund, Sweden

Myometrial tissue strips from term pregnant and non-pregnant guinea-pigs were studied in vitro by electrical field stimulation, and the adrenoceptor sensitivity to exogenously applied noradrenaline as determined The induced response was characterized by various sympatholytic and nerve blocking drugs. In previously contracted strips from non-pregnant animals field stimulation provoked a relaxation probably mediated via postganglionic adrenergic nerve terminals and myometrial β-receptors. In strips from myometrial tissue surrounding fetuses, no response was seen after field stimulation. Relaxation induced by exogenous noradrenaline was more pronounced and provoked at lower concentrations in pregnant than in non-pregnant tissue However in non-fetus-carrying uterine horns in unilateral pregnancies field stimulation still induced relaxation, and the sensitivity to exogenous noradrenaline was less than in the contra-lateral fetus-carrying horn. The physiological findings were compared with the endogenous level of uterine noradrenaline transmitter and the presence of adrenergic nerves, demonstrated by the Falck-Hillarp fluorescence technique It is suggested that the altered effects at term pregnancy are due to pregnancy induced postganglionic denervation of the adrenergic terminals in the fetus-carrying uterine horn. This is accompanied by an increased postsynaptic adrenoceptor sensitivity to the transmitter in analogue with denervation supersensitivity after adrenergic denervation. Functionally the present results verify the recent structural and biochemical findings of an adrenergic nerve degeneration in fetus-carrying myometrial tissue during pregnancy probably representing very special neuro-endocrine neuron-target relationship

Key words Myometrium, field stimulation, receptor sensitivity denervation

terus from many species including man is ed with adrenergic nerves located both d blood vessels and in association with bun- f smooth muscle cells (see review by Marshall The adrenergic nerves are derived from both cal long adrenergic neurons with their dies located pre or paravertebrally and from adrenergic neurons located very close to the e tissue (Owman, Sjöberg & Sjöstrand 1974). g advancing pregnancy the level of uterine ergic transmitter is very highly reduced, prob- due to structural degeneration of the adren- nerve plexus (Owman et al. 1975 Thorbert 1979). At the time of parturition this is re d by the absence of tyrosine hydroxylase ac tivity a rate-limiting noradrenaline synthesizing enzyme (Alm et al 1979) and the absence of the adrenergic axonal transmitter uptake mechanism (Alm et al. 1979 *b*) Further during advancing pregnancy ultrastructural signs appear characteristic of adrenergic nerve degeneration (Sporrong et al 1978).

By the stimulation of extrinsic nerves to the uterus motor responses can be elicited. These are the result of preganglionic nerve stimulation due to the occurrence of parasympathetic ganglia and short adrenergic neurons located very close to the uterus (see review by Marshall 1970). On the other hand by electrical field stimulation of myometrial tissue strips in combination with pharmacological

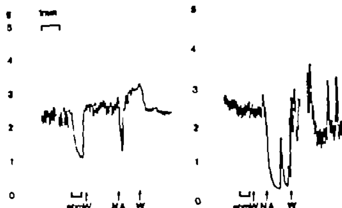


Fig. 1. Responses of spontaneously contracted myometrial strips from guinea-pig with unilateral pregnancy to electrical field stimulation (*stim*) and noradrenaline 20 μg/ml (*NA*). Left: strip from the empty uterine horn, right: strip from the contralateral fetus-carrying horn mounted in the same bath. At *W* the bathing solution was renewed 3 times.

taken from fetus-carrying horns in 7 out of 9 ant guinea-pigs and in strips from 4 animals eated with 6-OH-DA there was no response to ical stimulation.

...ition of noradrenaline caused relaxation of the pregnant (fetus-carrying) and the non-preg- myometrium, the threshold concentrations lower and the responses greater in the preg myometrium (Table 1). In strips from 2 non- ant animals the initial relaxation was followed small contraction which was abolished after ...ition of dihydroergotamine, 6 μg/ml.

...e responses to electrical stimulation and to ...ous noradrenaline administration of myo- ...al strips from animals with unilateral preg- ...are shown in Fig. 1. Electrical field stimula- ...id not produce any effect in the fetus-carrying ... whereas in the empty non-fetus-carrying ... a relaxant effect was seen. Further the addi- ...of noradrenaline produced relaxation in myo- ...al strips of both types of horns. However the ...ts were more marked in the strips from fetus- ...ing horns than in the strips from empty horns.

...USSION

...e present study electrical field stimulation of ...racted myometrial strips from estradiol-17β ...eated animals produced relaxant effect. Short stimulation pulses were used in order to avoid direct stimulation of the muscle cells (cf. Swedin 1971). The response was completely abolished after the administration of tetrodotoxin, suggesting that it was nerve-mediated. The total abolishment of the field stimulation-induced relaxation after the administration of guanethidine, being a potent adrenergic neuron blocking drug (see Costa 1966, Abbs & Dodd 1974) or in animals pre-treated with 6-OH DA, well-known for its ability to selectively degenerate adrenergic nerves (Thoenen & Tranzer 1968), strongly suggests that in the field stimulation experiments the nerves mediating the response are adrenergic. This is in line with previous histochemical findings, demonstrating adrenergic but no certain cholinergic nerve fibres in the guinea pig

Table 1. *Threshold concentrations*, μg/ml and inhibitory responses, g, to 20 μg/ml of noradrenaline in the guinea-pig uterus in vitro

Values are mean ± S.E., number of animals, *P*=significance

	Non pregnant	Pregnant (fetus-carrying)		*P*
Threshold concentration	18.5±4.5	5.0±0.9	4	<0.05
Response	0.3±0.1	2.5±0.4	7	<0.01

ceptor studies the relationship between the post ganglionic nerve terminals and their postsynaptic receptors can be more specifically studied This has been done in human and rat myometrial strips (Nakanishi et al 1969 Hollingsworth 1975) In view of the previous structural and biochemical findings obtained from guinea pig studies suggesting a pregnancy induced degeneration of the uterine adrenergic nerves during advancing pregnancy the present study on the uterine adrenergic nerve muscular relationship in non-pregnant and pregnant guinea pigs was undertaken for a functional comparison

MATERIALS AND METHODS

Experimental procedure Female sexually mature virgin guinea-pigs (400–500 g b wt.) were used They were given a single s c dose of estradiol-17β dissolved in pea nut oil (100 μg/250 g b wt) two days before sacrifice 8 4 and 1 day before killing, half the number of animals were given an i v injection of 6-OH-dopamine (6-OH DA) dissolved in ice cold saline containing 0.. mg/ml of ascorbic acid in respective doses of 100 mg/kg 100 mg/kg and 50 mg/kg. The animals were killed under a light ether anesthesia and the uterine horns dissected out From one horn some minute tissue pieces were rapidly taken for the fluorescence histochemical demonstration of adrenergic nerves according to the method of Falck and Hillarp (for details see Björklund et al 1972) and thereupon the whole uterine horn was quickly put into ice cold 0 4 N perchloric acid for the determination of the tissue content of noradrenaline according to the method of Bertler et al (1958) as modified by Häggendal (1963) The other uterine horn was put into ice cold Krebs-Ringer bicarbonate buffer (pH 7 4) immediately after the dissection and some minutes later myometrial tissue slices (about 6–9 mm in length 2–3 mm in width and 0 2 mm n thickness) were prepared as described by McIlwain & Rodnight (1962) using a razor blade and a cold object slide As judged from control sections examined in routine staining the slices obtained consisted of bundles of smooth muscle cells from the outer longitudinal smooth muscle layer Myometrial tissue slices were also prepared from pregnant guinea-pigs after some minute pieces of uterine tissue had been taken for fluorescence histochemistry of adrenergic nerves Most of the pregnant animals contained fetus(es) in both the uterine horns Two animals carried fetuses in only one of the uterine horns The time of pregnancy was about 60 days It was estimated by measuring the crown rump length and weight of the fetus(es) (Draper 1920 Kaufmann 1969)

The myometrial strips were mounted between platinum electrodes (5 mm apart) in an organ bath containing 30 ml of Tyrode solution (NaCl 0.8 KCl 0 02 $CaCl_2$ 0 02 $MgCl_2$ 0 01 $NaHCO_3$ 0.1 NaH_2PO 0 005 and glucose 0 1%) The solution was maintained at 37°C and aerated with a mixture of 95% O_2 and 5% CO_2. After mounting the strips had to equilibrate in the organ bath for about 3 h before experiments were undertaken. The tension of the [illegible] metrial strips was measured isometrically by [illegible] strain gauge transducers and recorded on a [illegible] nlscribe servo-recorder The initial tension was [illegible] g The strips then contracted spontaneously or were [illegible] tracted by adding metacholine or bradykinin [illegible] to the organ bath raising the tension to [illegible] For [illegible] cal field stimulation a Grass stimulator [illegible] square pulses with a duration of 2 ms, a frequency [illegible] Hz and of supramaximal voltage was used

In every expt. a myometrial strip from a [illegible] guinea-pig or from a non-pregnant [illegible] with 6-OH DA and a strip from a non [illegible] were studied simultaneously mounted in the same bath In two expts. using animals with unilateral [illegible] cy a strip from the empty (non-fetus-containing) [illegible] horn and a strip from the contralateral fetus-containing [illegible] from the same animal were studied in the same [illegible]

Statistics Student's *t*-test was used The [illegible] probability was accepted as significant.

Drugs The following substances were used [illegible] 17β 6-OH-dopamine metacholine chloride, [illegible] triacetate noradrenaline bitartrate dihydroergot[illegible] methansulphonate propranolol hydrochloride, [illegible] ethidine bisulphate hexamethonium bromide [illegible] toxin. The drugs were injected directly into the [illegible] Concentrations given are final bath concentrations [illegible] tive substance

RESULTS

Histochemistry In virgin animals only [illegible] tradiol 17β there was a moderate occurrence [illegible] energic myometrial nerves as well among the [illegible] dles of smooth muscle cells as around blood [illegible] sels in accordance with previous findings [illegible] 1968 Thorbert et al 1977) However no [illegible] myometrial nerves could be seen after the [illegible] bined treatment of estradiol 17β and 6-OH-DA [illegible] pregnant animals whether the uterine horn [illegible] tained fetuses or not

Tissue content of NA 6-OH DA produced [illegible] pronounced reduction of the endogenous [illegible] measured per uterine horn about 95% [illegible] to animals given only estradiol-17β [illegible] 0 305±0 052 μg to 0 015±0 001 μg [illegible] mean $n=4$ $P<0$ 001)

Stimulation experiments Electrical field [illegible] tion caused relaxation of the uterus from [illegible] nant guinea pigs reducing the tone from [illegible] to 1 4±0 1 g (mean ± S E $n=14$), i e by 30% ($P<0$ 001 paired comparisons). The [illegible] sponses were not affected by previous add[illegible] hexamethonium 300 μg/ml but were [illegible] abolished by guanethidine 3 μg/ml propr[illegible] μg/ml or tetrodo[illegible] g/ml In exp[illegible]

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uterus (Thorbert et al 1977) It should be inferred that in the guinea pig uterus there have recently been demonstrated populations of nerves which compared to the adrenergic nerves occur in a less amount and with a somewhat different intramural distribution and further contain intraneuronal peptides such as substance P or vasoactive intestinal polypeptide (VIP) (Alm et al 1977 1978 Larsson et al 1977) The uterine functions mediated by these peptidergic nerves are however unknown The unchanged field stimulation induced relaxation after the administration of hexamethonium indicate that the nerves are postganglionic adrenergic fibres From a functional point of view this is important to strengthen as histochemical studies have demonstrated that the guinea pig uterus is supplied both with long and short adrenergic neurons with their ganglionic cell bodies located respectively at a distance from or very close (even intramurally) to the organ (Sjöberg 1968 Thorbert et al 1977) The complete blockade of the field stimulation induced response after the administration of propanolol suggests that the nerve induced relaxation is mediated via β receptors

It has earlier been demonstrated that during advancing pregnancy the level of uterine adrenergic transmitter is highly reduced At the time for parturition no adrenergic nerves can be demonstrated histochemically and almost to noradrenaline transmitter can be demonstrated by quantitative fluorometric analyses (Sjöberg 1968 Owman et al 1975 Thorbert et al 1979) The almost completely abolished axonal transmitter uptake mechanism (Alm et al 1979*b*) the absence of activity of the noradrenaline synthesizing enzyme tyrosine hydroxylase at term pregnancy (Alm et al 1979*a*) in combination with the occurrence of ultrastructural signs of adrenergic terminal degeneration during advancing pregnancy (Sporrong et al 1978) suggest that the absence of uterine noradrenaline transmitter in the fetus-carrying horn is due to degeneration of the adrenergic nerve plexus This may in the present study be verified functionally by the almost total absence of a field stimulation induced response in myometrial strips from fetus-carrying uterine horns Further at the time for parturition the threshold concentration to get a noradrenaline induced response is lower and the responses induced are greater compared to strips from non-pregnant myometrial tissue Thus in the fetus-carrying uterine horn the pregnancy induced adrenergic nerve degeneration is also accompanied by increased postsynaptic adrenoceptor sensitivity to the noradrenaline transmitter This phenomenon could correspond to the well-known denervation supersensitivity described in other organs after adrenergic nerve denervation (see review by [illegible]lin & Trendelenburg 1972)

Also in the empty horn in unilateral pregnancy the level of noradrenaline transmitter was highly reduced at the time for parturition as [illegible] from previous histochemical and quantitative fluorimetrical studies (Owman et al 1975, Thorbert et al 1979) However in contrast to the fetus-carrying uterine horn the adrenergic nerve plexus of the empty horn and the cervix seemed to be structurally intact as the fluorescence microscopic pattern of the uterine adrenergic nerve plexus was restored after in vitro incubation in the presence of a [illegible] noradrenaline and further there still seemed to be a substantial and effective axonal noradrenaline transmitter uptake mechanism at term pregnancy (Alm et al 1978*b* Thorbert et al 1979) [illegible] the present material is small the findings [illegible] myometrial strips of empty uterine horns [illegible] monstrate a distinct relaxatory response [illegible] by electrical field stimulation and a moder[illegible] laxatory response after the administration of [illegible]ogenous noradrenaline On the contrary [illegible] from the contralateral fetus-carrying uterine [illegible] no response could be evoked by field stim[illegible] Taken together these findings might [illegible] previous assumption (see above) of a more [illegible] structurally intact adrenergic innervation of [illegible] empty uterine horn still able to evoke a [illegible] response It should be inferred that the [illegible] share of the total neuronal noradrenaline [illegible] to evoke a physiological response is [illegible] small (cf Lundborg 1967) This may expl[illegible] present field stimulation induced response [illegible] strips of the empty horn having a very low [illegible]mitter level

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ier mechanisms for neurotransmitter monoamines
ie choroid plexus

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LINDVALL, M HARDEBO J E & OWMAN CH. Barrier mechanisms for neurotransmitter monoamines in the choroid plexus Acta Physiol Scand 1980 108 215–221 Received 28 May 1979 ISSN 0001-6772 Departments of Histology and Neurology University of Lund S eden.

Studies were performed on choroid plexuses from mouse and rat. The plexus tissue sympathectomized to avoid interference from adrenergic nerves, is able to accumulate -DOPA as ell as NA and 5-HT against concentration gradient. Within the plexus tissue these compounds are metabolized by aromatic L-amino acid decarbo ylase MAO and COMT whose activities were determined radiometrically On the basis of this and the fate of systemically administered L-DOPA and DA studied by fluorescence histochemistry it is suggested that the epithelium of the choroid plexus provides not only structural but also an enzymatic barrier for transmitter amines. The enzymatic barrier for amine precursors is considerably less sufficient at this blood-CSF interphase The trapping of monoamines resembles the enzymatic barrier present in the endothelial cells and pericytes at the microvascular level of the blood-brain interphase.

Key ords Choroid plexus, blood CSF barrier amines and amine precursors MAO COMT

xchange between the circulation and the brain chyma must be strictly regulated in order to tain an adequate function of the brain In order ccomplish this, the interphase between the l and brain intercellular fluid compartment is pped with morphological barrier properties, lo- l at the level of the endothelial cell lining in the els within the brain tissue (Reese & Karnovsky Westergaard & Brightman 1973 van Deurs) and also at the level of the epithelium of the oid plexus (see Brightman 1975). If substances to pass beyond the choroid plexus they would ble to freely enter the brain parenchyma from ventricular ystem The barrier has a selective acter so that several substances may pass the branes of the luminal cellular lining of the brain ovessels—i.e the endothelial cells and peri- s—and the plexus epithelial cells by various s of transport mechanisms This is the case for monoamine transmitters DA NA, adrenaline, 5-HT in the direction from the brain to the ulation (Hardebo & Owman 1979) and for its recursor precursors e.g. L DOPA in both direc ns (Bertler et al. 1966, Wade & Katzman 1975 Hardebo et al. 1979 for review see Hardebo & Owman 1979*b*) Therefore, in order to assist in the barrier functions at the blood-brain interphase these cells must possess an efficient intracellular breakdown mechanism for such substances In the cerebral microvessels this breakdown is effectuated by enzymes present within the endothelial cells and pericytes, namely AAD and MAO leading to a conversion of these substances into inactive metabolites (for review see Hardebo & Owman 1979*b*)

The present study was undertaken to elucidate the possible presence of a corresponding enzymatic barrier mechanism at the specialized component of the blood-brain interphase constituted by the choroid plexus. Since the microvessels in the plexus are porous in that they have fenestrated endothelia, which permit the ready passage of solutes the

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Abbreviations used AAD aromatic L-amino acid decarboxylase BBB, blood-brain barrier COMT catechol-O-methyltransferase; CSF cerebrospinal fluid; DA, dopamine DOPA, 3 4-dihydroxyphenylalanine 5-HT 5-hydroxytryptamine MAO monoamine oxidase NA noradrenaline



...*g. 1* Fluorescence photomicrographs of mouse choroid ...uses and adjacent brain parenchyma. 160 (*a*) L ...A (50 mg/kg) following MAO inhibition. A diffuse fluorescence has accumulated in the plexus, and the ...llary walls of the brain parenchyma are intensely ...rescent. (*b*) DA (500 mg/kg). An intense green fluores... is found in the wall of plexus blood vessels and in ...adjacent connective tissue of the basement membrane. ...e plexus epithelium and the brain parenchyma are es...ially non-fluorescent. (*c*) DA (500 mg/kg) following ...treatment with nialamide. An intense fluorescence is found also in the epithelium of the choroid plexus.

...hyde reaction was that of the noradrenergic ...es (Edvinsson et al. 1974, 1975, Lindvall et al ...78, Lindvall 1979), running in between the plexus ...thelium and the wall of the underlying blood ...sels. Mast cells, which in mice and rats contain ...HT visible by the formaldehyde method, were ...ally not found in the plexuses.

Following systemic administration of L DOPA or ... pronounced fluorescence microscopic changes ...monstrable by the formaldehyde technique oc...rred in the choroid plexuses.

A green fluorescence seen after administration of ...DOPA was diffusely distributed in the vessel walls ...d in the epithelium. The intensity was low at the ...mg/kg dose; it was enhanced at increasing doses ...d was high at 160 mg/kg. Pretreatment of the animals with nialamide before the L DOPA injection further increased the green fluorescence in the plexuses (Fig. 1*a*). Animals receiving the decarboxylase inhibitors, NSD-1015 or benserazide showed a slightly elevated green fluorescence compared to animals given L DOPA alone, though the distribution of the fluorophore in the plexus tissue was not different from that of the latter group of animals. Administration of DA also resulted in a green fluorescence of the choroid plexus, but the distribution was partly different from that obtained after treatment with L DOPA. As for L-DOPA, DA accumulated in the wall of the blood vessels and in the adjacent connective tissue of the basement membrane. However, only a weak, diffuse fluorescence was found in the epithelium. Taking the fluorescence at the 100 mg/kg dose for comparison, this was found to be distinctly less pronounced than the epithelial cell fluorescence induced by 50 mg/kg of L DOPA. The vascular and subepithelial fluorescence was considerably enhanced with increasing doses of DA (Fig. 1*b*). When the DA treatment was combined with MAO inhibition, a very bright fluorescence appeared also in the plexus epithelium (Fig. 1).

Quantitative estimations of enzyme activity. The observations are summarized in Table 1. AAD activity was found in the choroid plexuses from both mouse and rat. Sympathetic denervation reduced the activity considerably in rat but not in mouse. Treatment of mice with the decarboxylase inhibitor benserazide almost abolished the AAD activity (Table 2). A substantial MAO activity was found in the plexus tissue, most of it remaining after sympathectomy. The dose of the MAO antagonist, nialamide, used in the fluorescence histochemical studies (100 mg/kg) inhibited the enzyme activity almost completely (Table 2). The choroid plexus of rat also contained a high activity of COMT.

Histochemistry of MAO. Histochemical analysis on mice showed that the choroid plexus contained MAO as shown by an overall staining of the tissue, but the rather low precision of the technique did not reveal any particular association of the formazan deposits to the various cellular components of the tissue. Nialamide-treated mice did not show any visible MAO activity in their choroid plexuses.

Incubation of isolated choroid plexuses. An accumulation of radioactivity was obtained in the choroid plexuses after incubation of tissues from the rat in the presence of varying concentrations of

barrier aspects are therefore in this context focussed on the epithelial lining of the choroid plexus

MATERIAL AND METHODS

Animals The study was performed on choroid plexuses from adult animals 250 female NMRI mice (25 g b wt) and 123 male Sprague Dawley rats (200–250 g b wt) The animals had free access to standard pellet food and water In order to differentiate between neuronal and extraneuronal enzyme activity the perivascular sympathetic nerve plexus was eliminated by bilateral removal of the superior cervical ganglion under pembutal-ethyl ether anesthesia 10 days prior to the measurement in 25 mice and 76 rats The mice were killed by decapitation whereas the rats were killed under light ethyl ether anesthesia by perfusion through the left ventricle or the heart with 0 9% saline After perfusion and/or decapitation of the animals the brains were immediately removed and the choroid plexuses from the lateral ventricles dissected out In the mice in addition the plexus of the third ventricle as well as the brain (excluding the brain stem) were dissected out

Drug treatments Groups of mice (6–10 in each group) received the following i p injections of drugs dissolved in 0 9% saline (the time intervals between two types of injections and between the last injection and decapitation are given within parentheses)

I Normal untreated control animals

II L DOPA (Sigma) 10 50 or 160 mg/kg (20 min)

III Nialamide (Pfizer) 100 mg/kg (60 min)

IV Nialamide 100 mg/kg (60 min) and L DOPA 50 mg/kg (20 min)

V Ro 4-4602 (benserazide Roche) 50 mg/kg (30 min) or NSD 1015 (Smith Kline and French) 100 mg/kg (60 min)

VI Ro 4-4602 50 mg/kg (30 min) and L DOPA 50 mg/kg (20 min)

VII NSD 1015 100 mg/kg (60 min) and L DOPA 50 mg/kg (20 min)

VIII DA (dopamine hydrochloride Sigma) 100 or 500 mg/kg (20 min)

IX Nialamide 100 mg/kg (60 min) and DA 100 or 500 mg/kg (20 min)

Fluorescence histochemistry The brain preparations were frozen in a propane-propylene mixture at the temperature of liquid nitrogen freeze-dried and treated in gaseous formaldehyde (1 h at 80°C) according to the Falck Hillarp method embedded in paraffin in vacuo and sectioned at 6 μ thickness for fluorescence microscopy (for details, see Björklund et al 1972)

Chemical determination of enzyme activity The choroid plexuses were homogenized in 20–60 μl of 10 mM sodium or potassium phosphate buffer (pH 6.5–7 0) and aliquots were taken for radiometric estimation of AAD and MAO activity as described by Fonnum (1976) COMT activity (not measured in mice) was estimated according to the method described by Axelrod (1962) Choroid plexuses from 5 mice or rats were pooled for each determination of AAD/MAO and COMT activity respectively Each enzymatic assay was linear with respect to protein concentration and incubation time and performed at V_{max} conditions. Tissue protein was determined according to the method of Lowry et al (1951).

Histochemistry of MAO The tetrazolium method of Glenner et al (1957) was used for cellular localization of MAO in mice Cryostat sections (20 μm thick) of brain tissue containing the choroid plexus were incubated for 30–45 min at 37°C in phosphate buffer (pH 7.4) containing tryptamine (Regis) as substrate and Nitro BT as the tetrazolium salt Tissues from normal mice and from mice given 100 mg/kg of the MAO inhibitor nialamide 1 h before decapitation were used. The controls were obtained by omitting the substrate (tryptamine) from the incubation solution.

Incubation of isolated choroid plexuses plexuses from sympathectomized rats were plexuses were transferred to incubation vials ice-cold Krebs Ringer buffer solution (in mM) NaCl 118 KCl 4.5 $CaCl_2 \times 2H_2O$ 2.5, M 1 0 $NaHCO_3$ 25 KH_2PO_4 1 0 to which was mg/ml glucose and 0.2 mg/ml ascorbic acid solution was continuously aerated with 95% O_2 CO_2. The vials were placed in an incubation allowed to equilibrate for 20 min at 37°C before which was started by adding either NA 3×10^{-8} and 10 M 9 1 Ci/mmol New England 5-HT (L 5-HT-G-^{3}H creatinine sulphate M 8 Ci/mmol Radiochemical Centre Am DOPA (L DOPA 2,3-^{3}H 3×10^{-8} and 10^{-6} M Radiochemical Centre Amersham) to the lower concentrations tested roughly correspond normal circulating levels of NA 5-HT and DOPA Hardebo & Owman 1979*b*) and the higher to those ured in the circulation during stress and certain cal conditions and for DOPA in the medical Parkinson's disease The tissue preparations were bated in the presence of these compounds for 37°C The incubation was terminated by transferring tissue into ice-cold buffer solution The plexuses washed twice in isotope-free cold buffer each In order to further characterize the uptake were also incubated in the presence of and at 0°C Thus characterization was performed concentrations corresponding to physiological the circulation In the CSF these concentrations lower

Measurement of radioactivity After incubation choroid plexuses were weighed and transferred scintillation vials They were solubilized in 0.5 ml (Packard) and liquid scintillation counting was in 10 ml Instagel (Packard) as were appropriate (25 μl) of the incubation medium Quench were obtained according to conventional principles

Statistics Differences between mean values analyzed with the Student's *t* test for unpaired data

RESULTS

Histochemistry of DOPA and DA The choroid plexus was easily recognized in brain tissue specimens from untreated animals by its slight background fluorescence The only specific fluorescence in the plexuses induced by the



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Incubation of isolated choroid plexuses An accumulation of radioactivity was obtained in the choroid plexuses after incubation of tissues from the rat in the presence of varying concentrations of

Table 1 *Activities of the enzymes aromatic L-amino acid decarboxylase (AAD), monoamine [illegible] (MAO) and catechol-O-methyltransferase (COMT) in the choroid plexuses from intact [illegible] pathectomized (SyX) mouse and rat*

SyX was performed by bilateral removal of the superior cervical ganglion 10 days prior to determinations [illegible] blanks subtracted Mean values ± S.E.M Number of determinations within parenthesis. Statistical evaluation [illegible] to Student's t test 0 001<P<0 01 n.s. is non-significant

Animal	Plexus tissue analyzed	AAD nmol/mg prot/h	MAO nmol/mg prot/h	COMT nmol/mg prot/h
Mouse	Intact	1 00±0.11 (10)	123 1±17 ? (10)	
	SyX	0 96±0 11 (5) n s.	83 7±15 0 (5) n.s	
Rat	Intact	1 99±0.25 (4)	66.7±3 0 (3)	1 91±[illegible] 1(?)
	SyX	0.5 ±0 15 (3)	–	–

radioactive L NA L 5-HT or L DOPA as evidenced by a higher radioactivity than in the incubation medium (Table 3) It should be noted that this represents the net accumulation of radiolabelled compound as a result of uptake and subsequent loss during the washing step The accumulation was significantly reduced by incubation at 0°C and by blockade of Na /K -dependent ATPase activity with ouabain (Table 3)

DISCUSSION

The histofluorescence analyses and the incubation experiment showed that the choroid plexus is able to take up L DOPA against a concentration gradient involving an energy requiring mechanism The process may thus be similar to that found for the capillary endothelium in the brain (see Hardebo & Owman 1979*b*) Inhibition of the decarboxylation step in the formation of DA from L DOPA increases the circulating levels of L DOPA which was reflected in a higher fluorescence in the choroid plexus tissue of animals given L DOPA and pretreated with benserazide or NSD 1015 supporting th[illegible] amino acid is indeed taken up by the plexus [illegible] The observation of a slightly higher plexus fl[illegible] cence when L DOPA was given together w[illegible] MAO inhibitor—in a dose that efficiently [illegible] MAO in the plexus—suggests that a minor [illegible] (reflected only in a minor fluorescence increase) the fluorogenic material in the plexus [illegible] metabolized by MAO This would imply [illegible] small part of the L DOPA taken up has been [illegible] decarboxylated to DA through the activity of [illegible] (denervation-resistant) AAD found in the ple[illegible] should be pointed out however that the d[illegible] boxylation capacity is by far higher in the [illegible] vascular endothelium of the brain parench[illegible] where it appears to constitute an important co[illegible] nent in the enzymatic BBB mechanism (for [illegible] see Hardebo & Owman 1979*b*) When DA [illegible] ven an intense green fluorescence was noted [illegible] wall of the plexus vasculature and also bey[illegible] vessel wall in the connective tissue Howev[illegible] epithelium contained only small amounts [illegible] amine which would indicate that the amine [illegible]

Table 2 *Activities of L-amino acid decarboxylase (AAD) and monoamine oxidase (MAO) in the [illegible] choroid plexus before and after treatment with the AAD inhibitor benserazide (50 mg/kg i.p [illegible] the MAO inhibitor nialamide (100 mg/kg i p 60 min)*

Mean values ± S E. number of determinations within parenthesis. Student's t-test P<0 001

Enzyme	Treatment: Control (nmol/mg prot/h)	Benserazide (nmol/mg prot/h)	Nialamide (nmol/mg prot/h)
AAD	1.28±0.11 (5)	0 0?±0 01 (6)*	0.9 ±0.2_ (6)
MAO	153 8±19.9 (5)	15 8±33 4 (6)	83±0 77 (6)

3 Net accumulation of radioactivity (tissue/medium ratio) following incubation of sympathec- d rat plexus tissue in the presence of either ³H-L-NA H L 5-HT or H L DOPA at two concentra- t 37°C and the effect of low temperature or ouabain

and	Concentration (M)	37°C	0°C	Ouabain 10^{-4} M	Concentration (M)	37°C
	3 10^{-8}	4 46±0 37 (6)	0.52±0.13 (6)	1 47±0.19 (4)	1×10⁻	2.91±0.3? (5)
	1 10^{-6}	5 60±0 44 (6)	0 41±0 10 (6)	1 91±0.29 (4)	1 10⁻	3.23+0.16 (4)
A	3 10^{-8}	3 05±0.33 (6)	0.83±0.26 (6)	1.21_0.17 (4)	1 10^{-6}	1.86±0.19 (4)

degree has entered the epithelium, or that it en efficiently degraded to non-fluorescent lites. The latter alternative is supported by h MAO activity found histochemically and ally in the plexus tissue (see also Lindvall & n 1979). It is also supported by the consider crease in fluorescence intensity when DA ven after MAO inhibition (although a compo- n this increment is probably the higher con- tion of circulating DA due to prevention of ation in peripheral tissues). That amines are ntly taken up into the plexus epithelium was ly shown in the incubation experiments, sup- g the findings by Tochino & Schanker (1965).
r concentration of neurotransmitter mono- es and their precursors in the brain extra- ar compartment has to be controlled within w limits in order to maintain an adequate func of the neuron systems. One aspect of this is the ated exchange across the blood-brain inter e (Wade & Katzman 1975 Hardebo & Owman b), this also involves transport processes in the oid plexuses, which constitute specialized re s of the cerebrovascular system. The choroid uses represent the main source for the forma- of CSF (Davson 1967 Dohrmann 1970 Cserr 1 1975 Pollay 1975). The plexuses receive a l-developed supply of adrenergic sympathetic es (Edvinsson et al 1974 1975 Lindvall et al. l, Lindvall 1979) which have an inhibitory ef on the production of CSF (Lindvall et al 1978) the course of the histochemical studies on the ymatic BBB for certain monoamines and their nediate precursor amino acids (Bertler et al 6) it was found that the fluorogenic amino acids l amines to varying extent accumulated in the roid plexus The findings of a sympathetic influ- ce on choroid plexus functions have initiated a ewed interest in the handling of neurotransmit n and related amines in the choroid plexus, and its position in the barrier system for such compounds at the interphase between the blood and CSF

Blood-borne molecules are restrained from entering the CSF at the level of continuous apical bands of tight junctions surrounding and sealing the epithelial cells (see Brightman 1975) The underlying capillary endothelium functionally resembles the leaky regions of the BBB, molecules and ions may pass across the cellular fenestra, between adjacent endothelial cells which are connected only by the discontinuous type of tight junctions, and possibly also by vesicular transendothelial transport (Brightman 1975).

In brain microvessels, and also in brain arterioles and pial vessels, an enzymatic barrier mechanism for these monoamines and also for their immediate precursors exist (Bertler et al 1966)—besides the morphological barrier in parenchymal vessels represented by the continuous tight junctions between adjacent endothelial cells, in combination with paucity of micropinocytotic vesicles (Reese & Karnovsky 1967) The enzymatic barrier involves an uptake into not only the endothelium but also the smooth muscle cell layer of the vessel walls followed by breakdown of the amine by MAO and COMT the precursor is first decarboxylated within the endothelium to the corresponding amine (see Hardebo & Owman 1979*b*) The presence of a similar enzymatic barrier to the amines at the blood-CSF interphase is suggested by the present findings of an energy-dependent amine uptake (see also Tochino & Schanker 1965) and a subsequent degradation within the choroid plexus

COMT is functionally related to the adrenergic receptor mechanism and has an extraneuronal localization in the autonomic innervation apparatus. Such localization of COMT can thus be assumed also in the choroid plexus. The MAO activity of the choroid plexus is both of the A and B

type (Lindvall & Owman 1979) there is reason to believe that the MAO A activity is primarily related to the sympathetic nerves present in the plexus In order to eliminate any error related to the presence of sympathetic nerves in the plexus tissue in the determinations of the amine uptake capacity sympathetic denervation was carried out beforehand in the incubation experiments Even though the perivascular nerve plexuses in the cerebrovascular system are traditionally not included among the components constituting the enzymatic BBB it is likely that the efficient neuronal transmitter inactivation mechanism represented by the adrenergic nerves will nevertheless assist in impeding the passage of sympathomimetic amines also at the level of the choroid plexus Apart from being involved in an enzymatic barrier mechanism functional studies have suggested that MAO participates in the regulation of an inhibitory tone—in which the sympathetic nerves are involved—on the CSF production (Lindvall & Owman 1979)

The enzymatic mechanism at the blood-CSF interphase may function for the passage of monoamines across the choroid plexus in both directions i e to prevent the passage of circulating monoamines into the CSF and thereby the brain extracellular fluid compartment, and also to assist in the metabolism of amines present in the CSF The metabolites formed by the enzymatic degradation of neurotransmitter monoamines such as homovanillic acid and 5-hydroxyindolic acid are excreted from the CSF to the circulation across the choroid plexus (Neff et al 1967 Ashcroft et al 1968 Cserr & van Dyke 1971 Bass & Lundborg 1976)

Although L DOPA is actively taken up into the choroid plexus epithelium (after its local passage through the capillary walls) the subsequent decarboxylation is only slight in comparison with that occurring in the endothelium of the BBB Other enzymes might be involved such as transaminase or COMT though this is not consistent with the intense epithelial fluorescence seen after L DOPA administration since the products formed (dihydroxyphenylacetic acid and 3-O-methyl DOPA) are not fluorogenic in the formaldehyde reaction (see Björklund et al 1972) It is therefore probable that the uptake of L DOPA is primarily a reflection of the transport into the choroid plexus of amino acids participating in the protein synthesis The choroid plexus thus seems to constitute an inefficient barrier for L DOPA in a similar way as the p[illegible] (Hardebo & Owman 1979b)

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racterization of the in vitro uptake of monoamines brain microvessels

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HARDEBO J E & OWMAN CH Characterization of the in vitro uptake of monoamines into brain microvessels. Acta Physiol Scand 1980, 108, 223–229 Received 28 May 1979 ISSN 0001-6772. Departments of Histology and Neurology University of Lund Sweden

The ability of brain microvessels to take up and metabolize transmitter monoamines (DA, NA, A and 5-HT) was studied in tissues from several species (rat, rabbit, guinea-pig, cat dog, baboon man) in vitro. Following incubation slices were analyzed by fluorescence histochemistry and isolated microvessels by measurement of radioactivity from tritiated amine Provided the MAO activity as inhibited trong accumulation, similar for all animals tested, occurred in the wall (endothelial cells and pericytes) of capillaries and venules—but not in larger vessels—of all species The degree of amine uptak and accumulation and the conditions under which it was inhibited suggested that it reflected saturable energy-dependent process with characteristics of both the extraneuronal and neuronal type of uptake processes The mechanism may serve as an inactivation of transmitter monoamines at the blood–brain interphase thereby assisting in the control of transmitter levels in the cerebral extracellular compartment

Key ords Blood–brain interphase brain microvessels, neurotransmitter monoamines, MAO

existence of a morphologic barrier between the d and brain parenchyma is well documented review see Rapoport 1976) Another aspect of barrier function is the presence of an enzymatic d-brain barrier for monoamine precursors, such L-3 4-dihydroxyphenylalanine (L DOPA) and hydroxytryptophan, first shown by Bertler et 1966) using fluorometric assay and fluorescence ochemical techniques. Systematically administ d L-DOPA and L 5-HTP are taken up into the l (endothelial cells and pericytes) of cerebral rovessels and is subsequently decarboxylated the corresponding amine i.e. dopamine (DA) 5-hydroxytryptamine (5-HT). Using the same hniques it has been found that only minor ounts of circulating neurotransmitter mono- ines, such as DA, noradrenaline (NA) adrena- (A) and 5-HT accumulate within the endo- lial cells and pericytes of cerebral microves- (Hardebo et al 1979): the bulk of mono- ines is prevented from leaving the brain cir lation already at the luminal membrane of the endothelial cells (Bertler et al 1966 Hardebo et al 1979*a*) Evidence has been presented for the exist ence for an enzymatic blood–brain barrier not only for the monoamine precursors but also for the monoamines themselves they are rapidly broken down by monoamine oxidase (MAO) present in the endothelial cells and pericytes of the brain micro- vessels (Bertler et al 1966, Spector Baird-Lam- bert & Lai 1977 Hardebo et al. 1977 and 1979*b*) and in addition by MAO and catechol-O-methyl transferase (COMT) in the smooth muscle cell layer of pial vessels and brain parenchymal arterioles (Hardebo et al. 1979*b*) On the other hand, little is known about the clearance of neurotransmitter monoamines in direction from the brain parenchyma to the circulation It can be assumed that the local presence of a sufficient excretory mechanism into the cerebral microcirculation—along with the reup- take into the aminergic nerve terminal and the clearance from the cerebrospinal fluid—will assist in the control of the level of the amine in the brain extracellular compartment In the present study a

brain microvascular uptake of various neurotransmitter monoamines is demonstrated and characterized

MATERIAL AND METHODS

Animals The study was performed on adult animals of either sex namely 64 Sprague Dawley rats 29 albino rabbits 7 guinea-pigs 5 cats 4 dogs 4 baboons and brain tissue from 5 adult female and male patients The laboratory animals had free access to food and water These animals were killed under Nembutal anesthesia by perfusion of the vascular system with 0 9% saline to eliminate any error caused by the presence of blood cells The brains were dissected out and kept in Krebs Ringer buffer solution on ice (for composition see below) or during preparation of microvessel fractions in cold phosphate buffer The human tissue (macroscopically intact frontal and temporal cortex) was obtained during neurosurgical lobe resection operations the tissue was immediately placed in ice-cold buffer solution and transported on ice to the laboratory (within 30 min)

Incubation of tissue slices Approximately 1 mm thin slices of the parietal cortex (frontal and temporal cortex in man) caudate nucleus cerebellum hypothalamus (at the level of the median eminence) spinal cord and heart from the various animals were cut with a razor blade and transferred to incubation vials containing ice-cold Krebs-Ringer buffer solution The buffer solution had the following composition (mM) NaCl 118 KCl 4 5 $CaCl_2 \times H_2O$ 2 5 $MgSO_4 \times 7H_2O$ 1 0 $NaHCO_3$ 25 KH_2PO_4 1 0 to which was added 1 mg/ml glucose 0 mg/ml ascorbic acid and 0 05 mg/ml EDTA

The vials were placed in an incubation bath at 37°C for preincubation during 20 min in the presence of the MAO inhibitor nialamide (10 M) followed by incubation during 20 min after the addition of various doses (1 10 or 100 µg/ml) of NA A DA or 5 HT In addition slices from the parietal cortex of rat were incubated in the presence of several increasing doses (1 5 10 20 50 or 100 µg/ml) of NA in an attempt to find an upper limit for the uptake process The buffer solution was continuously aerated with a mixture of 95% O_2 and 5% CO_2 giving pH of about 7 4 and a pO_2 around 200 mmHg (as measured on an MRK II blood gas analyzer Radiometer Copenhagen) Slices from the parietal cortex (frontal and temporal cortex in man) were also incubated with NA A DA or 5 HT (1 or 10 µg/ml) in the absence of nialamide Control slices were incubated without addition of any drugs

In order to further characterize the uptake the incubations of the parietal cortex (frontal or temporal cortex in man) with monoamines were also performed in the presence of either cocaine (10 or 10 M) desmethylimipramine (10^{-4} or 10^{-4} M) phenoxybenzamine (10 or 10^{-4} M) oestradiol-17 β (10^{-4} or 10^{-4} M) theophylline (10 M) ouabain (10^{-4} or 10^{-} M) or dinitrophenol (5 × 10 or 10^{-3} M) These compounds were added to the bath at the start of the preincubation together with nialamide Incubation was also performed in a low Na /high K milieu (in the buffer solution KCl substituted for all NaCl) and under a combination of anoxic conditions (continuous

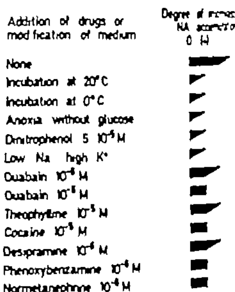


Fig 1 Accumulation of NA fluorophore in the walls of microvessels in rat cortical slices incubated in the presence of 10 µg/ml noradrenaline following MAO inhibition (10 M nialamide) and processed according to the formaldehyde histofluorescence method The degree of vascular fluorescence intensity above the diffuse general background fluorescence is expressed arbitrarily the *tapering part of the bars* indicating intermediary intensities

bubbling with 95% N_2 and 5% CO_2) and deprived of energy substrate (glucose) and also at 20°C and 0°C

Fluorescence microscopy Following the incubation the various tissue pieces were frozen to the temperature of liquid nitrogen and further processed for fluorescence monoamine histochemistry according to the Falck Hillarp method (Björklund Falck & Owman The paraformaldehyde used in the histochemical procedure had previously been equilibrated with air of humidity The formaldehyde-induced fluorescence visible fluorophores of NA A and DA are indistinguishable in the sense that they have the same spectral characteristics and exhibit a green light under the optical conditions used whereas 5 HT exhibits a yellow rapidly fading light (Björklund et al 1972) Fluorescence microscopy was carried out on 6 µm thick sections in a Leitz fluorescence microscope equipped with an Osram HBO 200 mercury lamp and Schott BG 12 and OG 4 primary and secondary filters respectively The relative fluorescence intensity in the microvessel walls was estimated according to an arbitrary scale (see Fig 1) using preparations The different preparations to be compared after incubation at the various conditions (see below) were always freeze-dried and further processed simultaneously

Isolation of cerebral microvessels Whole brains of rat and rabbit were used After removal they were put in ice-cold 67 mM phosphate buffer (pH 7 4) throughout the procedure (see Hardebo et al 1979) meninges including the pi brane and its vessels

-efully torn off and the choroid plexuses were ... White matter including the whole brain stem, ... ected away. Tissue obtained from 4-6 rats or 2-3 ... as chopped ... th a razor blade, and then gently ... aspirated by some 10 strokes up and dow... through ... ferent 10-20 ml plastic ... yringes equipped with ... (pore size 1000 μm, 500 μm and 280 μm, respec... fixed to their open cut end. The material was ... ently homogenized by hand ... th a loosely fitting ... ettle in ... smooth glass tube (0.1 mm clearance) ... mogenate ... as washed through one nylon sieve ... μm pore size. The material remaining on the ... ter extensive washing ... was re-homogenized and ... 3-4 times through the same ... carefully washed ... he tissue passing these sieves was collected and ... hrough another sieve with 75 μm pore size. The ... remaining on this sieve after washing was re-... ized and re-sieved in the same ... carefully washed ... he tissue fraction remaining on the last sieve ... after ... e washing consists of capillaries ... venules and a ... er vessels ... hereas most of the larger vessels and ... branching off into clusters of small vessels, as well ... small clusters of neurons and glia, remain on the ... sieve (Hardebo et al. 1979b). Neurons, glia, small ... mel segments and subcellular fragments pass the ... eve. The capillary fraction is pure with regard to ... and is only contaminated with ... few glial endfeet ... to the vessel wall (Hardebo et al. 1977). The yield ... vessels is less than 1/1000 of the original wet ... of the grey matter. Brendel, Meezan & Carlson ... ave show ... that a mixed fraction of intracerebral ... prepared under similar conditions ... is metabolical-... e.

...ation of isolated microvessels. The microvessel ... s were transferred to incubation vials containing ... l Krebs-Ringer buffer solution (composition as ...). The vials were placed in an incubation bath and ... l to equilibrate for 20 min at 37°C before incuba-... ... was started by adding ³H-NA to the solution ... me preparations ... ere incubated for 15 min at ... ations of 37°C. The buffer solution was continuous-... ated ... th 95% O_2 and 5% CO_2. The incubation was ... ated by transferring the tissue into ice-cold buffer ...

The microvessel fraction was collected by cen-... ation at 4°C at 110 g and washed twice in isotope-free ... buffer for each 10 min.

...rder to further characterize the uptake microvessel ... were also incubated in the presence of ouabain ... M), under combined anoxia and absence of glucose ... at 0°C.

...asurement of radioactivity. After incubation, the ... vessel) samples ... ere weighed and transferred to ... scintillation vials. They ... ere solubilized in 0.5 ml ... (Packard), and liquid scintillation counting was ... med in 10 ml Instagel (Packard), as ... ere appro-... samples (25 μl) of the incubation medium. Quench ... ctions ... ere obtained according to conventional pro-...

...ugs. Dopamine hydrochloride (Sigma), ...-noradrena-... bitartrate monohydrate (Sigma), L-adrenaline bitar-... (Sigma), 5-hydroxytryptamine creatinine hyd-... oride (Sigma), cocaine hydrochloride (ACO), oua-... bain (Sigma), 2,4-dinitrophenol (Chroma), theophylline (Sigma), desmethyl imipramine (Desipramine, Geigy), phenoxybenzamine hydrochloride (Dibenzyline, Smith Kline and French), nialamide (Pfizer) and L-norepinephrine 7-³H (New England Nuclear 9.1 Ci/mmol).

Statistics. Mean values were compared according to the Student's *t*-test for unpaired data.

RESULTS

Incubation of tissue slice. Fluorescence microscopy of the various brain regions from control slices incubated in buffer solution alone showed a dense network of delicate catecholamine-containing nerve terminals emitting a green fluorescence. In the cerebellum only a small number of isolated green-fluorescent axons were present. The parenchymal blood vessels were equipped with varying amounts of sympathetic nerves. In the heart tissue adrenergic nerves were seen in the myocard and in association with the blood vessels. All tissues showed a slight diffuse non-specific greenish background fluorescence. The vessel wall proper was in all regions essentially non-fluorescent, except for the intense autofluorescence of the internal elastic membrane of larger pial arteries.

After incubation with the various monoamines an uptake (as reflected in an accumulation of fluorophore well above the diffuse background fluorescence) was seen in the brain microvessel walls (endothelial cells and pericytes of capillaries and small veins) in all species studied. The intensity of the general background fluorescence was also increased. No clearcut accumulation above the diffuse background fluorescence had occurred in the walls of the brain parenchymal arterioles or heart vessels. Possibly a slight uptake and accumulation was seen in the wall of pial arteries. Inhibition of MAO by nialamide clearly enhanced the accumulation of the monoamines in the microvessel wall, as evidenced by a stronger fluorescence intensity in relation to the background fluorescence. At an identical concentration in the incubation bath DA, NA and A all induced a similarly strong fluorescence in the microvessel wall, whereas the fluorescence induced by 5-HT was weaker. This discrepancy in fluorescence intensity is at least partly explainable by the varying fluorescence yield in the standard formaldehyde reaction (Björklund et al. 197?). An upper limit of fluorescence intensity in brain microvessel walls could be disclosed when the tissue

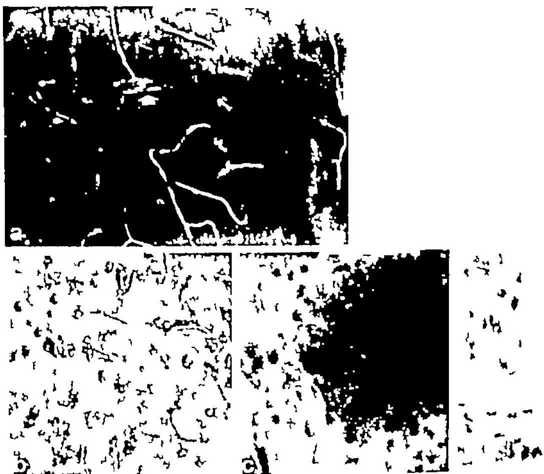
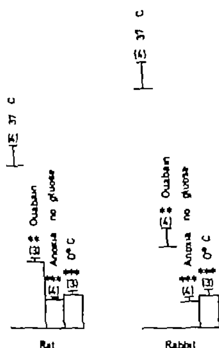


Fig Fluorescence photomicrographs of brain tissue slices following incubation in the presence of catechol- previous inhibition of MAO (10⁻ M in the medium) (a)–(c) Human temporal cortex ×90 (a) Intense fluoresc microvessel walls but no accumulation in arterioles (arrow) after incubation with 10 µg/ml noradrenaline presence of ouabain 10 M the degree of noradrenaline accumulation is markedly reduced (c) During hypoxia only very little noradrenaline accumulates in the microvessel walls. (d)–(e) Rat parietal cortex (d) Intense fluor in the walls of capillaries and venules but not in arterioles (arrow) after incubation with 10 µg/ml dopamine (e) Desipramine (10^{-4} M) reduces the microvascular accumulation so that only the more voluminous nuclear are clearly visible ×75

slices pretreated with nialamide were incubated in the presence of increasing concentrations of NA a maximum fluorescence was seen at the 20 µg/ml concentration It has been shown in model experiments that there is a linear relationship between fluorescence intensity and the catecholamine concentration Due to quenching of the fluorescence at high catecholamine concentrations a further increase in the concentration above a certain level gives an unchanged or even slightly diminished intensity (Ritzén 1966 Jonsson 1971) However our finding most probably reflects an upper limit for accumulation in the microvessel wall rather than being the result of quenching, which occurs only at very high local concentrations

The inhibitory influence on the uptake of N various drugs or by modification of the medi listed in Fig 1 and illustrated in Fig. – The of inhibition was the same for the other amines studied except for 5-HT which was resistant to desipramine treatment

Incubation of microvessels An uptake cumulation of radioactivity was obtained in the microvessel fraction after incubation in the pre of tritiated NA as evidenced by a conside higher radioactivity than in the incubation me (Fig. 3) The uptake was significantly reduce blockade of Na /K -dependent ATPase with ouabain by incubation under anoxi absence of glucose or g 3)



...4 Trace/medium ratio (T/M mg tissue per µl ...) following incubation of isolated cerebral micro... in the presence of ³H-NA under control condi... °C), and in the presence of ouabain (10^{-4} M), ...mbined anoxia and deprivation of glucose, or at ...perature (0°C). Values are means S.E. number ...iments within parenthesis. Control vs. experi... according to Student' t-test. $0.001<p<0.01$ ...001

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...resent study provides evidence for the exist... in various species including man of a satu... energy-dependent uptake mechanism for ...transmitter monoamines in the endothelial and pericytes of brain microvessels in con... to the endothelium of brain parenchymal ar... es. For comparison, in e.g. the erythrocyte ...mines enter by facilitated diffusion (Blakeley ...ical 1978). The present study does not show ...her the uptake occurs across the luminal or the ...minal side of the microvessel wall or both. An ...se of monoamines into brain microvessel walls ...rat has been reported by estimation of for...ldehyde-induced fluorescence following incuba... of brain DA, NA and their alpha-methylated ...ogues (Hamberger 1967). In the latter study it ...found that the uptake was prevented by des...hylimipramine d-amphetamine and cocaine to a similar extent as the uptake into NA-storing nerve terminals of reserpine-pretreated animals.

The efficiency of the ³H NA uptake as reflected by the ratio of radioactivity between the microvessel fraction and the medium was of the same order as that reported for the uptake into sympathetic nerves under similar conditions (Edvinsson & Owman 1977, Alm et al. 1979) but considerably higher than the extraneuronal uptake in smooth musculature (Alm et al. 1979). The energy-dependency of the microvessel uptake was confirmed by the inhibition accomplished by hypothermia, combined anoxia and glucose deprivation or uncoupling of the oxidative phosphorylation with dinitrophenol. The results from the incubations with low sodium and high potassium as well as in the presence of ouabain suggest that the uptake is linked with a Na/K-dependent ATPase. The uptake was inhibited both with compounds that are known to affect the neuronal (cocaine desipramine phenoxybenzamine) and extraneuronal (normetanephrine, oestradiol, phenoxybenzamine) transport of monoamines. This shows that, although the accumulation in brain microvessels is extraneuronal it has several features in common with the axonal uptake process however without the subsequent cytoplasmic retention of the amine by a reserpine-sensitive storage mechanism (Bertler et al. 1966). Thus the uptake and accumulation of amines in the cerebral microvessels in many respects resembles the uptake shown to occur in the wall of pulmonary microvessels (for review see Gillis 1976) though a difference in the degree of uptake between the various amines could not be established.

The extraction of trace-amounts of neurotransmitter monoamines from the brain circulation during a single capillary passage is only about 3–5% (Oldendorf 1971, Hardebo et al. 1977, Hardebo & Nilsson 1979). Only at high circulating concentrations and after inhibition of the MAO activity is it possible by fluorescence microscopy to visualize a weak accumulation of monoamines in the brain microvessel wall (Hardebo et al. 1979b). The poor penetrability across the luminal endothelial membrane is due to the unity of the structural blood–brain barrier (Reese & Karnovsky 1967) which to a large extent impedes the passage of these water soluble and polar substances. On the other hand, when the blood–brain barrier is opened experimentally an uptake and accumulation of monoamines in

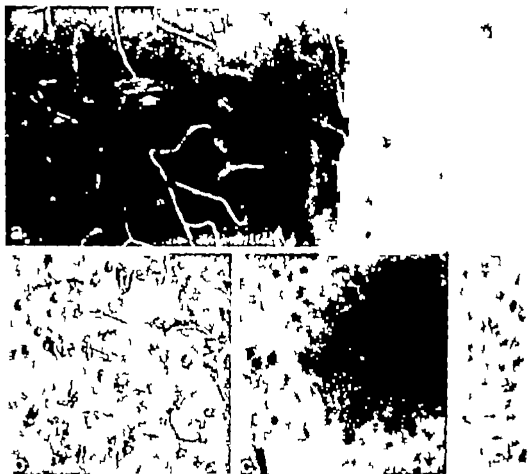


Fig 2 Fluorescence photomicrographs of brain tissue slices following incubation in the presence of catechol[illegible] previous inhibition of MAO (10 M in the medium) (*a*)–(*c*) Human temporal cortex ×90 (*a*) Intense fluor[illegible] microvessel walls but no accumulation in arterioles (arrow) after incubation with 10 μg/ml noradrenaline [illegible] presence of ouabain 10⁻ M the degree of noradrenaline accumulation is markedly reduced (*c*) During hypox[illegible] only very little noradrenaline accumulates in the microvessel wall (*d*)–(*e*) Rat parietal cortex (*d*) Intense fluor[illegible] in the walls of capillaries and venules ( ) but not in arterioles (arrow) after incubation with 10 μg/ml dopamine (*e*) Desipramine (10^{-4} M) reduces the microvascular accumulation so that only the more voluminous [illegible] clearly visible ×75

slices pretreated with nialamide were incubated in the presence of increasing concentrations of NA a maximum fluorescence was seen at the 20 μg/ml concentration It has been shown in model experiments that there is a linear relationship between fluorescence intensity and the catecholamine concentration Due to quenching of the fluorescence at high catecholamine concentrations a further increase in the concentration above a certain level gives an unchanged or even slightly diminished intensity (Ritzén 1966, Jonsson 1971) However our finding most probably reflects an upper limit for accumulation in the microvessel wall rather than being the result of quenching which occurs only at very high local concentrations

The inhibitory influence on the uptake of [illegible] various drugs or by modification of the med[illegible] listed in Fig 1 and illustrated in Fig. 2 The [illegible] of inhibition was the same for the other [illegible] amines studied except for 5-HT which [illegible] was resistant to desipramine treatment

Incubation of microvessels An uptake a[illegible] cumulation of radioactivity was obtained in t[illegible] crovessel fraction after incubation in the pr[illegible] of tritiated NA as evidenced by a consi[illegible] higher radioactivity than in the incubation [illegible] (Fig 3) The uptake was significantly red[illegible] blockade of Na /K -dependent ATPase [illegible] with ouabain by incubation under an[illegible] absence of glucose or [illegible] Fig 3)

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the cells of the microvessel wall is clearly distinguishable (Flodmark et al 1969 Hardebo et al 1979a) Under these conditions the monoamines may enter the cytoplasm of the endothelial cells by pinocytosis (Hansson Johansson & Blomstrand 1975) or it may pass between (or through) the endothelial cells to reach the abluminal side of the endothelial membrane and from here enter the endothelial cell as well as the pericyte It can be assumed that a sufficient inactivating mechanism for extraneuronal monoamines in the brain parenchyma is of fundamental value for maintaining an adequate brain function a clearance process via the brain microvessel walls may work as a local complement to the neuronal re uptake mechanism and enzymatic breakdown It is therefore not unexpected that the active uptake of monoamines into the microvessel walls is almost exclusively working across the abluminal membrane of the endothelial cell in direction from the brain into the cytoplasm of this cell (and of the pericyte) This would explain why monoamines following intraparenchymal (Bertler et al 1966) or intraventricular infusion (Fuxe & Ungerstedt 1968 and own unpublished observations) accumulate in the microvessel walls in a narrow zone around the stich channel and periventricularly respectively

Pretreatment with the MAO inhibitor nialamide enhanced the in vitro accumulation of monoamine into the endothelial cells and pericytes of the microvessel wall This finding offers further support for the presence of MAO in these cells (Bertler et al 1966 Spector et al 1977 Hardebo et al 1977 and 1979b)

The study has shown the presence of a saturable energy-dependent transport of monoamines into brain microvessel walls The process shares characteristics with both neuronal and extraneuronal monoamine uptake mechanisms and resembles the microvascular transport of NA and 5-HT in the lung Once entering the microvessel wall the monoamines are effectively metabolized by the locally present MAO Although monoamines penetrate the luminal membrane of the cerebral microvessel only to a minor extent MAO will provide an inactivation of those who do enter However the major inactivation mechanism at the level of the microvessel is accomplished by the transport and metabolism of amines in the direction from the brain parenchyma Both aspects of this inactivation at the blood-brain interphase may assist in securing an adequately low level of neurotransmitter [illegible] extracellular compartments However [illegible] pathological conditions—marked impair[illegible] morphologic barrier capacity and brain [illegible] anoxia—this inactivation mechanism [illegible] cient The transient increase in neurotr[illegible] monoamine levels that occurs in the brain [illegible] lular fluid compartment initially during [illegible] anoxia (Meyer et al 1973 1974 see [illegible] & Zervas 1974)—that may be detrimental [illegible] brain by increasing the metabolism [illegible] chemic-anoxic brain area—may in part be [illegible] impaired uptake of the transmitter [illegible] microvascular wall locally

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...phonylurea (Glibenclamide) enhances somatostatin ...inhibits glucagon release induced ...rginine

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EFENDIĆ, S. ENZMANN F NYLÉN A. UVNÄS-WALLENSTEN K. & LUFT R Sulphonylurea (glibenclamide) enhances somatostatin and inhibits glucagon release induced by arginine Acta Physiol Scand 1980 108. 231 233 Received 5 June 1979 ISSN 0001-6772. Department of Endocrinology Karolinska Hospital Department of Pharmacology Karolinska Institute Stockholm, Sweden and Hoechst Aktiengesellschaft, Medizinische Abteilung, Frankfurt, West Germany

Arginine significantly stimulated the release of insulin glucagon and somatostatin from the isolated perfused rat pancreas A sulphonylurea, glibenclamide markedly enhanced the effect of arginine on somatostatin release and inhibited its effect on glucagon release Insulin release was not modulated by addition of glibenclamide These findings support the idea of a paracrine interaction of islets hormones.

Key ords Somatostatin, glucagon insulin, perfused rat pancreas

...s been generally accepted that the principle ...of action of the sulphonylureas is through ...tion of insulin release from the pancreatic (Loubatières 1946, Grodsky et al 1967 Cerasi 1969 Loubatières et al 1970 Malaisse et al ...). Recently this opinion was seriously ques...d, and it was suggested that the chronic an...betic action of the sulphonylureas could be ...ibled to one or several extrapancreatic effects ...e drug (Lebovitz et al 1977 Feinglos & Lebo... 1978) Moreover it has been demonstrated that ...honylureas administered in vivo to humans and ...t increase the number of insulin receptors and ...ordingly enhance insulin sensitivity (Feinglos Lebovitz 1978 Soretnen 1978) In addition ...e authors report that sulphonylureas suppress ...agon release which could explain, at least par...y the antidiabetogenic action of these drugs ...mols et al. 1969 Laube et al 1970). The latter ...ing could not be confirmed by other inves...ators (Pek et al 1977 Baumeister et al 1975) The present study demonstrates that one sul...phonylurea, glibenclamide suppressed arginine induced glucagon release which was most likely the result of enhanced release of somatostatin—the third pancreatic hormone

MATERIAL AND METHODS

The experiments were performed on isolated perfused rat pancreas. The perfusate Krebs-Ringer bicarbonate solution to which was added 0.8 g/l of glucose and 20 g/l of beef albumin was administered into the coeliac artery and run into the prepared pancreas by an open circuit. The flow-rate of the perfusate was 2.5 ml/min. Insulin was determined by double-antibody radioimmunoassay (Hales & Randle 1963) using insulin reagent kits (Radiochemical centre Amersham) and a rat insulin standard Glucagon was assayed by the charcoal separation technique using 30K antibodies (Aguilar Parada et al 1969). Somatostatin was measured by a radioimmunoassay using own antibodies (R 141E) (Efendić et al. 1978) The sensitivity of this assay was about 5 pg/ml Cross-reactivity of the antibody was less than 0.01% with insulin, glucagon substance P LH-RH vasopressin and oxytocin The antigenic specificity of the antibodies was determined using somatostatin analogues (Arimura et al 1978).

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enhancement of somatostatin release. This s a paracrine action of somatostatin. Such a hesis calls for an explanation of the absence of tion of insulin release in our experiments.

present, we can visualize two alternatives y that glibenclamide enhances arginine in- l insulin release and that this is then count- ed by somatostatin the net effect being un- d arginine induced insulin release. Secondly in the rat pancreas the somatostatin produc- -cells are closely connected with the glucagon ucing A-cells but not with the B-cells, the rine action of somatostatin would predomin- be exerted on the A-cells. The latter alterna- s supported by the finding that addition of anti- tostatin serum to rat islets markedly stimu- glucagon but not insulin release (Barden et al.). The present findings suggest that inhibition ucagon release may be one of the modes of of sulphonylureas and that somatostatin l be the mediator of this inhibition. The signifi- e of such an action in human diabetes remains studied. Nevertheless our findings in the el system used may open new approach in the ch for oral antidiabetic drugs.

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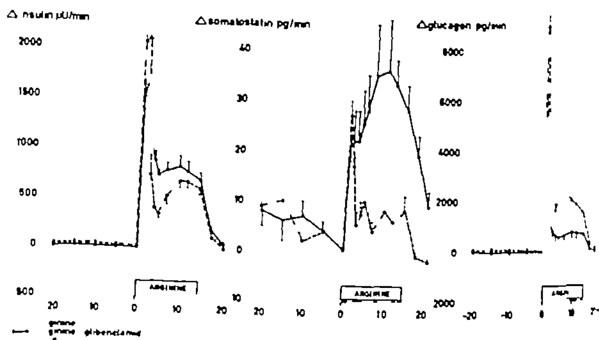


Fig 1 Effect of arginine in the absence (O- O) and presence (●—●) of glibenclamide on the release of in[illegible] somatostatin and glucagon from the isolated perfused rat pancreas. The pancreases were equilibrated for 20 min [illegible] administration of arginine and glibenclamide The values are Means ± S E of eight parallel experiments

RESULTS

Administration of arginine (5 mg/ml) significantly enhanced insulin glucagon and somatostatin release from the pancreas (Table 1) The release pattern was biphasic for all three hormones (Fig 1) Addition of glibenclamide (1 µg/ml) significantly enhanced the effect of arginine on somatostatin release but inhibited it effect on glucagon rele[illegible] Insulin release was not significantly altered

DISCUSSION

We believe that the inhibition of arginine [illegible] glucagon release by glibenclamide can be [illegible]

Table 1 *Effect of Glibenclamide on arginine induced release f insulin glucagon and somatostat[illegible] isolated perfused rat pancreas (mean ± S E n=8)*

Response*	Arginine	Arginine + glibenclamide	P
Insulin			
0–6 min	6 ?01±496	6 947±389	NS
7 15 min	4 919±494	6 117±700	NS
0–15 min	11 121±990	13 065±974	NS
Glucagon			
0–6 min	?0 166± 966	10 ?09±1 ?9?	<0 01
7–15 min	15 864± 683	5 967±1 09?	<0 005
0–15 min	36 030±5 629	16 176±2 315	<0 01
Somatostatin			
0–6 min	76±14	170±4?	NS
7 15 min	5 ±17	?67±5	<0 005
0–15 min	1 8±?9	436±9	<0 01

Integrated responses for insulin in µU × min × ml for glucagon and somatostatin [illegible] × ml

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enal medullary control of muscular and hepatic cogenolysis and of pancreatic hormonal retion in exercising rats

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W have previously shown that in exercising adrenodemedullated rats chemically sympathectomized with 6-hydroxydopamine, the muscular and hepatic glycogen breakdown were diminished and the plasma glucagon and insulin concentrations were lower and higher respectively than in controls. T elucidate whether these effect could be ascribed selectively to the lack of the adrenal medulla or to the lack of the sympathetic nerve endings, rat were either surgically adrenodemedullated, chemically sympathectomized with 6-hydroxydopamine both adrenodemedullated and chemically sympathectomized or shamtreated 3 weeks after adrenodemedullation liver biopsy and cardiac blood was obtained Subsequently the rats either rested or swam with a tail weight for 100 min. Immediately afterwards, cardiac blood was drawn and liver and muscle tissue were collected. The effects of combined adrenodemedullation and chemical sympathectomy were found to be due to the lack of the adrenal medulla Furthermore in adrenodemedullated rats, compared to controls the concentration of norepinephrine in plasma was markedly reduced during exercise The total activity ($a+b$) of glycogen phosphorylase in muscle was reduced 20% by adrenodemedullation. The findings indicate that during prolonged exercise adrenomedullary hormones enhance muscular and hepatic glycogenolysis as well as glucagon secretion but inhibit insulin secretion in the rat. Furthermore, in contrast to the findings in man, in the rat major part of the circulating norepinephrine is of adrenomedullary origin during exercise

Key ords Exercise, epinephrine norepinephrine sympathetic nervous system, muscles, liver glycogen, glucagon, insulin, glycogen phosphorylase

have previously shown (Galbo et al. 1978) that renodemedullation combined with chemical mpathectomy alters the metabolic and hormonal sponses to exercise in rats. Thus muscular and patic glycogen breakdown were diminished and e plasma concentrations of glucagon and insulin ere lower and higher respectively compared control rats These findings raised the question as to what extent the differences between drenodemedullated and chemically sympathec omized rats versus control rats were due to either he lack of the adrenal medulla or to the lack of the peripheral sympathetic nerve endings. Further more adrenodemedullation inevitably damages some of the adrenal cortex. Accordingly the effects of adrenodemedullation might to some extent be due to decreased cortical function Finally the sympatho-adrenal system might be of importance for the turnover of muscular enzymes Thus the effect of adrenodemedullation and/or chemical sympathectomy might be exerted through a decrease in glycogenolytic enzyme concentrations in muscle since these procedures were carried out two weeks before the acute exercise test.

In the present study we have tried to elucidate to what extent the effects of combined adre

1 The effect of exercise adrenodemedullation and 6-hydroxydopamine on hepatic and muscular ... gen concentrations (expressed as mmol of gluco e kg wet weight) and on concentrations of lactat l l⁻) and glucos (mmol l⁻) in blood

... are mean ± S.E. The number of observations is shown in parenthesis. Rats were exercised (exercise) or resting ...), adrenodemedullated (DM) or sham-operated (DM⁺) and treated with 6-hydroxydopamine (6-OHD⁺) or ... treated (6-OHD⁻). The hepatic glycogen concentration and the blood glucose concentration were measured both ... and after rest and exercise in every rat, whereas the remaining variables were measured only after exercise or ... lent period of rest. The concentrations of both hepatic glycogen and of glucose in blood before exercise or ... lent period of rest were similar in all groups of rats, and averaged ($\bar{X}$ ± S E.) 415±8 n=66 and 5 76±0 06, n=66, ... ively For each variable all mean values differ significantly from each other (p<0 05) except for mean values ... ated by If values in DM and DM groups and/or in 6-OHD and 6-OHD⁻ groups did not differ significantly ... were pooled

	Exercise		Exercise	
	DM	DM	DM	DM
...ase in hepatic glycogen				
...HD⁺	164±14	41±14	85±10	
...HD⁻	(17)	(16)	(33)	
...gen in superficial ...us muscle				
...HD⁺	42±1 (15)	73 2 (8)	46±1 (16)	35±1 (17)
...HD⁻		28±2 (9)		
...gen in deep vastus muscle				
...HD⁺	38±1	19±1	46±1	35±1
...HD⁻	(17)	(17)	(16)	(17)
...gen in soleus muscle				
...HD⁺	43±1	18±1	44±*	29±2
...HD⁻	(17)	(17)	(16)	(17)
...te in blood				
...HD⁺	2 81±0.42	6 07±0 41	1.57±0 30	
...HD⁻	(16)	(17)	(31)	
...ase in blood glucose				
...OHD⁺	0 72±0 32* (25)		0 12±0.28* (33)	
...OHD		3 75±0.53 (9)		

...ficantly were pooled Therefore if nothing else is ...d, all the numbers in the tables for each variable are ...ficantly different (p<0 05) from each other

...SULTS

...control rats (sham-operated and sham-injected) ...f glycogen concentration in skeletal muscle had ...nificantly decreased after 100 min of swimming, ...d the decrease in the hepatic glycogen concentra...n was during exercise significantly larger than ...ring the equivalent period of rest (Table 1). Dur...g exercise in these rats the concentrations in ...asma of glucagon and cortisol and of glucose and ...ctate in blood increased (Tables 1 3 and 4), ...hereas the concentration of insulin in plasma did ...ot change significantly (Table 2) No consistent ...ffects of exercise adrenodemedullation or 6-hy...droxydopamine on the hematocrit or on the concentrations of FFA and glycerol in serum were found (not shown) Body weight was neither influenced by adrenodemedullation nor by chemical sympathectomy The mean was 270±2 g ($\bar{X}$ ±S E., n=67)

Effects of adrenodemedullation

In resting rats adrenodemedullation increased the concentrations of muscular glycogen (Table 1) and plasma insulin (Table), and decreased the concentration of glucagon in plasma (Table 3). During exercise the decreases in muscular and hepatic glycogen concentrations were markedly reduced by adrenodemedullation (Table 1) Furthermore, after exercise the concentrations of glucose and lactate in blood and of glucagon in plasma were lower and the concentration of insulin in plasma was higher in

nodemedullation and chemical sympathectomy could be ascribed to the lack of the adrenal medulla or to the lack of the peripheral sympathetic nerve endings Rats were either demedullated chemical ly sympathectomized both demedullated and sym pathectomized or sham treated and were either resting or exercised by swimming The application of an in vivo liver biopsy technique made it possible more accurately to determine the exercise induced hepatic glycogen breakdown The enzyme glycogen phosphorylase is of importance for muscular glycogen breakdown capacity Accordingly we measured the total activity $(a+b)$ of phosphorylase in muscle Finally the plasma concentration of cor tisol was measured as an indicator of adrenocortical function

MATERIALS AND METHODS

67 male Wistar rats weighing 180–?00 g were anesthe-tized with ether and either adrenodemedullated or sham-operated Adrenodemedullation was performed by elec trocoagulation after lumbar incisions No supplement of saline was given after demedullation In order to allow regeneration of the adrenal cortex the surgery was carried out ?1 days prior to the exercise test

In half of both the adrenodemedullated and the sham-operated rats chemical sympathectomy was accom-plished The procedure which has been found to re sult in the most complete destruction of adrenergic nerve terminals in rats (Thoenen & Tranzer 1968) was used Accordingly one week after the surgery each rat received two injections of 34 mg kg b w 6-hydroxydopamine dissolved immediately before injection in 0 5 ml 0 9% NaCl containing 0.5 mg ascorbic acid The two injections were given in a tail vein 6 h apart One week later the rats received further ?×68 mg kg b w 6-hydroxydopamine i.v In the other half of the rats only the solvent was injected

The exercise test was carried out in the morning and the rats fasted 1–2 h in advance During ether anesthesia 0 ml of blood were drawn by cardiac puncture for glucose and hematocrit analysis Then a small biopsy (15–40 mg) of the liver was obtained through a minute abdominal incision which afterwards was sutured Bleeding wa neg-ligible After 15 min of recovery the rats either swam 100 min with a tail weight of lead (? % of body weight) in water maintained at 33–34°C or they remained resting At the end of exercise or the equivalent period of rest the rat were quickly anesthetized with ether and 8 ml of blood were drawn by cardiac puncture Then samples of the liver of the superficial part of the va tus lateralis muscle (which consists predominantly of fast-twitch white fibers) of the deep part of the vastus lateralis muscle (predomi-nantly fast twitch red fibers) and of the soleus muscle (predominantly slow-twitch red fibers) were quickly re-moved and frozen in liquid nitrogen Blood and tissue sampling was completed within 5 min after exercise In adrenodemedullated rats the selective destruction of adrenal medulla was verified by light microscopy of hematoxylin and eosin stained sections of each adrenal gland

The methods of analysis of blood and tissue metabolites, hematocrit glucagon and catecholamines have been reported elsewhere (Galbo & Holst 1976 Galbo et al 1977a) Insulin was determined by radioimmunoassay using rat insulin as the standard. Within assay coefficient of variation ($n=70$) was 3% At a plasma level concentration (rat plasma) of 900 pmol l recovery of added insulin to final concentrations between 906 and 1[illegible] pmol l yielded values within ±5% of expected values Cortisol in plasma was determined by a commercially available solid phase radioimmunoassay kit (Mallinckrodt) Plasma from adrenalectomized rats yielded results which were not significantly different from zero Total $(a+b)$ phosphorylase activity in muscle was determined fluorometrically after incubation of the extract homogenate with purified rabbit muscle phosphorylase kinase and ATP (Lowry & Passonneau 1973) The activity was determined as formation of glucose 1-phosphate from glycogen added in excess, and was expressed as µmol of glucose 1-phosphate g wet muscle min [illegible] decrease in glycogen concentrations between liver samples taken before and after blood drawing might be underestimated due to differences in the blood content of the liver However the difference between the glycogen content in biopsies taken before and immediately after exsanguination in 6 rats was 1±5 ($\bar{x}$±S E) expressed as mmol of glucose kg wet weight

The concentration of glycogen in muscle was measured in wet tissue Thus changes in water content might have influenced the measured differences in glycogen concentrations. Therefore the water content was determined in biopsies of the deep part of the vastus lateralis [illegible] taken from 5 rats from each of the eight groups [illegible] samples were weighed before and after freeze drying [illegible] significant differences in water content between the groups were found

The major part of the data was analyzed by a three-way analysis of variance preceded by Bartlett's test for homogeneity of variance (Snedecor & Cochran 1965) [illegible] major discrepancies were found with Bartlett's test The three treatments (ways) are exercise adrenal demedullation and injection of 6-hydroxydopamine Each treatment has two levels Accordingly exercise and exercise denote that the rats were exercised and resting respectively DM and DM denote adrenodemedullation and sham-operation respectively and 6-OHD and 6-OHD denote injection with 6-hydroxydopamine and [illegible] injections respectively The plasma concentrations of norepinephrine epinephrine and cortisol were not measured in all groups of rats These data were analyzed by a two way analysis of variance (Snedecor & C[illegible] 1965) By the analysis of variance it is calculated [illegible] and to what extent a certain treatment or combination of treatments (interactions) influence the data. If a certain treatment or combination does not influence the data significantly the data can be pooled paying no attention to the tre tment or combination of treatments Accordingly for each v riable mean [illegible] h did not [illegible]

3 *The effect of exercise adrenodemedulla- ad 6-hydroxydopamine on the concentration -agon in plasma (pmol l⁻¹)*

were exercised (exercise) or resting (exercise), demedullated (DM⁺) or sham-operated (DM) and l with 6-hydroxydopamine (6-OHD⁺) or sham- l (6-OHD⁻) No interactions between the three ents were found. This fact allows data to be pre as one total mean and an estimated value for each effects.

mean	56±3
of treatments	
	−22_3
ercise	17±3
HD⁺	+ 9±3
l	22±3
ercise	−17_3
HD⁻	− 9±3

are mean ± S.E. The estimated mean of any is found by adding the appropriate estimated effects total mean. the mean concentration in the group exercise 6-OHD⁻ is 56−22+17 9=42±6. The on the result is √(4 3)² 6

bolic and hormonal responses to exercise reas the destruction of the peripheral sympathe- erve endings by chemical sympathectomy only slight effects. Thus in adrenodemedullated the exercise-induced muscular and hepatic ogen breakdown were markedly reduced and plasma concentrations of glucagon and insulin e lower and higher respectively compared to findings in control rats (Tables 1 2 and 3) In ping with the reduced exercise-induced muscu- and hepatic glycogenolysis were the findings of er post-exercise blood lactate and glucose con- trations in demedullated rats than in controls ble 1). In adrenodemedullated rats circulating nephrine was after exercise undetectable in 10 t of 13 rats and the plasma norepinephrine con- tration was markedly reduced (Table). These ts indicate both that the procedure of adre demedullation was efficient and that, in con- st to the findings in man (Vendsalu 1960) in the t a major part of the circulating norepinephrine is f adrenomedullary origin during exercise. We have reviously shown (Galbo et al 1978) that the con- ntrations of norepinephrine in muscle pancreas d liver are reduced to 27% 12% and 5% respec ely of control values by chemical sympathec omy

Other studies have demonstrated an only minor influence (Struck & Tipton 1974 Sembrowich et al 1974) or no influence at all (Gollnick et al 1970 Maling et al 1966) of adrenodemedullation on glycogenolysis in exercising rats The difference between these observations and the findings in the present study could possibly be due to differences in the state of training of the animals or in the applied exercise procedures or intensities Further more in the present study the application of a liver biopsy technique made it possible to determine the exercise-induced hepatic glycogen breakdown in individual rats. Accordingly we were probably able to measure hepatic glycogen breakdown more accurately than in other studies. In accordance with our results administration of β-receptor blocking agents to rats has been shown to decrease the hepatic glycogen breakdown during swimming (Kindler et al 1978) Furthermore infusion of the β-adrenergic blocking agent propranolol to steadi- ly exercising dogs decreased the rate of muscular glycogen breakdown and was accompanied by a marked fall in the plasma lactate concentration and by a more rapid decrease in the plasma glucose concentration in comparison with control experi- ments (Issekutz 1978) In keeping with these find- ings β-adrenergic blockade has been shown to di- minish the reduction in glycogen content in an in situ isolated, stimulated muscle preparation (Costin et al 1971).

In rats with an intact adrenal medulla chemical sympathectomy increased the exercise-induced muscular glycogenolysis in the fast-twitch muscle fibers (Table 1) This finding might be due to im- paired cardiovascular adjustments to exercise lead-

Table 4 *The effect of exercise and adrenodemedul- lation on the concentration of cortisol in plasma (nmol l⁻¹)*

Values are mean ± S.E The number of observations is show in parenthesis. Rats were either exercised (exer cise⁺) or resting (exercise⁻) and adrenodemedullated (DM⁺) or sham-operated (DM⁻). The mean values differ significantly ($p < 0.05$) from each other Exercise values did not differ significantly and were therefore pooled

	DM	DM
Exercise⁺	199±32 (7)	309±30 (8)
Exercise	119±21 (16)	

Table 2 *The effect of exercise adrenodemedullation and 6-hydroxydopamine on the plasma concentrations of insulin (pmol l⁻¹) norepinephrine (nmol l⁻¹) and epinephrine (nmol l⁻¹) and on the total (a+b) of glycogen phosphorylase in muscle (expressed as µmol of glucose 1 phosphate g wet wt min⁻¹)*

Values are mean ± S E The number of observations is shown in parentheses Rats were exercised (exercise) (exercise) adrenodemedullated (DM) or sham-operated (DM) and treated with 6-hydroxydopamine (6-OHD) or sham-treated (6-OHD⁻) For each variable the mean values differ significantly ($p<0.05$) from each other. If the exercise and exercise groups and/or in 6-OHD and 6-OHD groups did not differ significantly they were pooled

	DM		DM	
	Exercise	Exercise	Exercise	Exercise
Insulin in plasma 6-OHD 6-OHD	656±34 (33)		210±34 (33)	
Norepinephrine in plasma 6-OHD 6-OHD	6 2±2 3 (13)	Not measured	19.3±— (14)	Not measured
Epinephrine in plasma 6-OHD 6-OHD	Undetectable in 10 out of 13 rats	Not measured	11 9±1.— (14)	Not measured
Phosphorylase (a+b) activity in muscle 6-OHD 6-OHD	11 3±0 5 (26)		14.2±0.5 (29)	

adrenodemedullated rats than in controls (Tables 1 2 and 3)

The concentrations of norepinephrine and epinephrine were measured after exercise only Adrenodemedullation decreased the plasma concentration of epinephrine to below detection limit (less than 0 01 pmol in 50 µl plasma = 0 2 nmol l) in 10 out of 13 rats (Table 2) The three detectable values were 0 2 0 3 and 0 4 nmol l Furthermore demedullation reduced the norepinephrine concentration in plasma markedly (Table 2)

The plasma concentration of cortisol was at rest not significantly different in demedullated and in control rats (Table 4) After exercise an increase in the concentration of cortisol was found in both demedullated and in control rats The increase however was largest in control rats (Table 4)

The activity of total (a+b) glycogen phosphorylase in muscle was decreased 20% by adrenodemedullation (Table 2)

Effects of 6-hydroxydopamine (6-OHD)

The combination of adrenodemedullation and treatment with 6-hydroxydopamine did not produce more marked effects than did adrenodemedullation alone (Tables 1 2 and 3) In rats with an intact adrenal medulla, treatment with 6-OHD increased the exercise induced glycogen breakdown in the superficial part of the vastus lateralis muscle compared to the breakdown in control rats (Table 1) A similar tendency was found in the deep part of the vastus lateralis muscle (not shown) Furthermore destruction of the peripheral sympathetic nerve endings by 6-OHD in rats with an intact adrenal medulla abolished the exercise induced increase in blood glucose concentration (Table 1) In both adrenodemedullated rats and in rats with an intact adrenal medulla 6-OHD increased the concentration of glucagon in plasma at rest and after exercise (Table 3)

Treatment with 6-OHD did not significantly reduce the plasma concentration of norepinephrine (Table 2) However in rats with an intact adrenal medulla the ratio norepinephrine/epinephrine was decreased by 6-OHD [2 4±0.5 vs 1 7±0 9 ... S E $p<0.02$ t test)] due to a tendency toward both a lower concentration of norepinephrine ... and a higher concentration of epinephrine ... compared to controls

DISCUSSION

The present study has shown ... at in the rat adrenodemedullation has a ... influence

Table 3. The effect of exercise adrenodemedullation and 6-hydroxydopamine on the concentration of glucagon in plasma (pmol l⁻¹)

...re exercised (exercise⁺) or resting (exercise⁻), ...emedullated (DM⁺) or sham-operated (DM⁻) and ...ith 6-hydroxydopamine (6-OHD⁺) or sham- (6-OHD⁻). No interactions between the three ...ts were found. This fact allows data to be pre... as one total mean and an estimated value for each ...ffects

...mean	56±3
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	−22±3
...cise	+17±3
...D⁺	+ 9±3
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...cise	−17±3
...D⁻	9±3

...are mean ± S.E. The estimated mean of any ...found by adding the appropriate estimated effects ...total mean ... the mean concentration in the group ...exercise 6-OHD⁻ is 56−22+17 9=42±6. The ...the result is √(4 ...)=6

...bolic and hormonal responses to exercise ...es the destruction of the peripheral sympathe... ...erve endings by chemical sympathectomy only ...slight effects. Thus in adrenodemedullated ...of the exercise-induced muscular and hepatic ...ogen breakdown were markedly reduced and ...plasma concentrations of glucagon and insulin ...lower and higher respectively compared to ...findings in control rats (Tables 1, 2 and 3). In ...ping with the reduced exercise-induced muscu... and hepatic glycogenolysis were the findings of ...er post-exercise blood lactate and glucose con...trations in demedullated rats than in controls ...ble 1). In adrenodemedullated rats circulating ...ephrine was after exercise undetectable in 10 ...of 13 rats and the plasma norepinephrine con...tration was markedly reduced (Table 2). These ...lts indicate both that the procedure of adre...demedullation was efficient and that in con...st to the findings in man (Vendsalu 1960), in the ...a major part of the circulating norepinephrine is ...adrenomedullary origin during exercise. We have ...viously shown (Galbo et al. 1978) that the con...trations of norepinephrine in muscle, pancreas ...d liver are reduced to 77%, 1% and 5% respec...ely of control values by chemical sympathec...my.

...Other studies have demonstrated an only minor influence (Struck & Tipton 1974, Sembrowich et al. 1974) or no influence at all (Gollnick et al. 1970, Maling et al. 1966) of adrenodemedullation on glycogenolysis in exercising rats. The difference between these observations and the findings in the present study could possibly be due to differences in the state of training of the animals or in the applied exercise procedures or intensities. Furthermore, in the present study the application of a liver biopsy technique made it possible to determine the exercise-induced hepatic glycogen breakdown in individual rats. Accordingly, we were probably able to measure hepatic glycogen breakdown more accurately than in other studies. In accordance with our results administration of β-receptor blocking agents to rats has been shown to decrease the hepatic glycogen breakdown during swimming (Kindler et al. 1978). Furthermore, infusion of the β-adrenergic blocking agent propranolol to steadily exercising dogs decreased the rate of muscular glycogen breakdown and was accompanied by a marked fall in the plasma lactate concentration and by a more rapid decrease in the plasma glucose concentration in comparison with control experiments (Issekutz 1978). In keeping with these findings β-adrenergic blockade has been shown to diminish the reduction in glycogen content in an in situ isolated, stimulated muscle preparation (Costin et al. 1971).

In rats with an intact adrenal medulla chemical sympathectomy increased the exercise-induced muscular glycogenolysis in the fast-twitch muscle fibers (Table 1). This finding might be due to impaired cardiovascular adjustments to exercise lead-

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Values are mean ± S.E. The number of observations is shown in parenthesis. Rats were either exercised (exercise⁺) or resting (exercise⁻) and adrenodemedullated (DM⁺) or sham-operated (DM⁻). The mean values differ significantly ($p<0.05$) from each other. Exercise⁻ values did not differ significantly and were therefore pooled.

	DM⁺	DM⁻
Exercise⁺	199±37 (7)	309±30 (8)
Exercise⁻	119±21 (16)	

ing to insufficient muscular perfusion to the tendency towards increased plasma epinephrine concentrations intensifying β-adrenergic stimulation or to impaired mobilization of other substrates in sympathectomized rats compared to controls. If the mobilization of other substrates (e.g. fat) during exercise is impeded by sympathectomy the muscles probably derive more of the necessary energy from glycogen combustion (Rennie & Holloszy 1977).

Regarding the pancreatic hormonal secretion we found that the plasma insulin concentration was markedly increased by adrenodemedullation but uninfluenced by exercise and chemical sympathectomy (Table 2). Possibly the lack of steady state in the short period (less than 2 min) between the termination of exercise and blood drawing prevented the finding of a decreased plasma insulin concentration in exercised rats. However in control rats the unchanged insulin concentration during exercise indicates a suppression of insulin secretion since the blood glucose concentration increased in these rats (Table 1). Such a suppression was found in neither adrenodemedullated nor sympathectomized rats since the plasma insulin concentration as well as the blood glucose concentration in these rats were unchanged during exercise (Table 1). Thus also the sympathetic nerves seem to inhibit insulin secretion during exercise. Our findings are in keeping with the increase at rest and after exercise in the plasma insulin concentration found after administration of the α-receptor blocking agent phentolamine (Luyckx & Lefebvre 1974) and with the epinephrine induced decrease of the insulin secretion (Wright & Malaisse 1968) in resting rats. Destruction of sympathetic nerves by immunosympathectomy did in a previous study not influence plasma insulin and glucose concentrations during exercise (Luyckx et al. 1975). However in that study the work intensity and with that probably sympatho-adrenal activity was lower than in the present study and no sign of inhibition of insulin secretion was found in exercising control rats.

As to glucagon exercise and sympathectomy increased whereas adrenodemedullation decreased the plasma glucagon concentration (Table 3). The decrease in the plasma glucagon concentration due to adrenodemedullation probably reflects the reduction of β-adrenergic enhancement of glucagon secretion (Gerich et al. 1976, Luyckx & Lefebvre 1974). The increase in the plasma glucagon concentration found after chemical sympathec[illegible] in contrast to findings describing enhance[illegible] glucagon secretion by the sympathetic [illegible] (Gerich et al. 1976, Luyckx et al. 1975). Our fi[illegible] is not easily explained. However the [illegible] plasma glucagon concentration found with [illegible] ing exercise intensity during short term exe[illegible] man (Galbo, personal communication) [illegible] inhibiting influence of the sympatho-adrenal [illegible] upon the amount of glucagon reaching the [illegible] al blood stream. During exercise an increase [illegible] plasma glucagon concentration which could [illegible] ascribed to sympatho-adrenal influence was [illegible] (Table 3). Thus in the rat and more pro[illegible] man (Galbo et al. 1976b) the glucagon respo[illegible] exercise cannot entirely be ascribed to enh[illegible] ment by the sympatho-adrenal system. The [illegible] has been put forward that during exercise the p[illegible] ma glucose concentration influences glucagon se[illegible] retion (Galbo et al. 1977b, Luyckx & Lef[illegible] 1974). This is probably so during prolonged e[illegible] cise when a decrease in the plasma glucose [illegible] centration may enhance glucagon secretion ([illegible] et al. 1977b, Harvey et al. 1974). In the pre[illegible] study however a decline in the blood glucose [illegible] centration was not found during exercise. [illegible] cannot be excluded that the lack of steady [illegible] the short period after the end of exercise and [illegible] blood drawing prevented the demonstration of [illegible] decrease in the glucose concentration.

The findings in the present study imply that [illegible] ing prolonged exercise circulating catechol[illegible] originating in the adrenal medulla enhance mu[illegible] and hepatic glycogenolysis and glucagon secr[illegible] but inhibit insulin secretion. However it has [illegible] been clarified whether the catecholamines [illegible] enhance glycogen breakdown and glucagon se[illegible] tion during exercise or whether these effects [illegible] due to a primary inhibition of insulin secretio[illegible] insulin is able to inhibit glucagon secretion (C[illegible] et al. 1976) and to promote glycogen storage. [illegible] ably the reduced concentration of glucagon in [illegible] nodemedullated rats did not play any role fo[illegible] lower exercise induced hepatic glycogenol[illegible] This is so since during exercise the acti[illegible] glucagon on hepatic glycogenolysis seems [illegible] permissive. In exercising dogs and rats the he[illegible] glucose production may proceed unchanged [illegible] absence of an increase in the plasma glucagon [illegible] centration (Vranic et [illegible] 79, Galbo et [illegible] but i[illegible] decreased if [illegible] ca[illegible]

is markedly decreased (Issekutz & Vranic) The existence of an effect of circulating cholamines on enzyme turnover was indicated the finding of a 20% decrease in the total $(a+b)$ ogen phosphorylase activity in muscle in adre medullated rats compared to controls (Table This decrease however seems small in comparison to the effect of adrenodemedullation on ogen breakdown during exercise (Table 1)

The findings in the present study could hardly be to insufficient adrenocortical function in adre medullated rats since in these rats (1) a supment of saline was not necessary for survival and ght gain, (2) the hepatic and muscular glycogen centrations (Table 1) as well as the blood glue concentration (Table 1) were not decreased pared to controls, and (3) at rest the plasma centration of cortisol was not decreased comed to controls and the concentration increased ing exercise (Table 4).

Statistical analysis was carried out in collaboration ...ond stat Per Kragh Andersen, Statistical Research ... Danish Medical and Social Science Research Coun- The investigation as supported by grants from ...h Research Foundation, Idrettens Forskningsrad and ... Foundation 6-hydroxydopamine and the ... radioimmunoassay kit were kindly donated by ... and Byk-Mallinckrodt, respectively. Lisbeth Kall, ... Unk and Rikke Grønholt performed skilled ...al assistance.

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Regarding the pancreatic hormonal secretion we found that the plasma insulin concentration was markedly increased by adrenodemedullation but uninfluenced by exercise and chemical sympathectomy (Table 2) Possibly the lack of steady state in the short period (less than 2 min) between the termination of exercise and blood drawing prevented the finding of a decreased plasma insulin concentration in exercised rats However in control rats the unchanged insulin concentration during exercise indicates a suppression of insulin secretion since the blood glucose concentration increased in these rats (Table 1) Such a suppression was found in neither adrenodemedullated nor sympathectomized rats since the plasma insulin concentration as well as the blood glucose concentration in these rats were unchanged during exercise (Table 1) Thus also the sympathetic nerves seem to inhibit insulin secretion during exercise Our findings are in keeping with the increase at rest and after exercise in the plasma insulin concentration found after administration of the α-receptor blocking agent phentolamine (Luyckx & Lefebvre 1974) and with the epinephrine induced decrease of the insulin secretion (Wright & Malaisse 1968) in resting rats Destruction of sympathetic nerves by immunosympathectomy did in a previous study not influence plasma insulin and glucose concentrations during exercise (Luyckx et al 1975) However in that study the work intensity and with that probably sympatho-adrenal activity was lower than in the present study and no sign of inhibition of insulin secretion was found in exercising control rats

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The findings in the present study imply that during prolonged exercise circulating catecholamines originating in the adrenal medulla enhance muscle and hepatic glycogenolysis and glucagon secretion but inhibit insulin secretion However it has not been clarified whether the catecholamines directly enhance glycogen breakdown and glucagon secretion during exercise or whether these effects are due to a primary inhibition of insulin secretion as insulin is able to inhibit glucagon secretion (Clarke et al 1976) and to promote glycogen storage Probably the reduced concentration of glucagon in adrenodemedullated rats did not play any role for the lower exercise induced hepatic glycogenolysis This is so since during exercise the action of glucagon on hepatic glycogenolysis seems to be permissive In exercising dogs and rats the hepatic glucose production may proceed unchanged in the absence of an increase in the plasma glucagon concentration (Vranic et al 1979 Galbo et al 1979), but is decreased if the plasma glucagon concentra-

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lterations in membrane electrical properties ring long-term denervation of rat skeletal muscle

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SELLIN L. C. & THESLEFF S. Alterations in membrane electrical properties during long-term denervation of rat skeletal muscle Acta Physiol Scand 1980, 108. 243–246. Received 8 June 1979 ISSN 0001-6772. Department of Pharmacology University of Lund Sweden

Some membrane electrical properties of the extensor digitorum longus muscle of the rat were examined up to 21 days after denervation. The resting potential was significantly more depolarized at 3 days after denervation than it was at later times. The rate of rise (dV/dt) of the action potential decreased throughout the time course of the study but approached steady value between 14 and 21 days after denervation. In addition, the dV/dt of tetrodotoxin (TTX)-resistant action potentials increased up to and including 7 days after nerve section, but declined thereafter When expressed as % of control, the dose-response to TTX was similar throughout denervation. It is suggested that the large depolarization observed early in denervation may be related to the turnover of membrane constituents occurring at this time. The results also suggest that denervation produces reduction in the number of fast TTX-sensitive Na channels with the appearance of new population of slow TTX-resistant channels. However long term denervation results in reduction in the density of both types of channels.

Key words Denervation, membrane electrical properties, sarcolemma, skeletal muscle

t well known that denervation of mammalian letal muscle produces profound changes in whose electrical properties. Among these alter ns are: a depolarization of the resting potenl decrease in the rate of rise (dV/dt) of the ion potential and the appearance of tetrodotoxin (TTX)-resistant action potentials (Albuque & Thesleff 1968 Redfern & Thesleff 1 1971b). The impetus for the present study e from the observation that after an initial inse in the dV/dt of TTX-resistant action potentials early in denervation, the toxin-resistance peared to decrease between 5 and 12 days after ervation (Redfern & Thesleff 1971b) The possility remained that the appearance of TTX-resistant Na channels could be a temporary response d may not persist during long-term denervation. erefore, the present study was undertaken to exine the effects of long-term denervation on some the membrane electrical properties of rat skelel muscle

ETHODS

ll experiments ere conducted in vitro on the extensor itorum longus muscle (EDL) of adult male rats (250–350 g) of the Sprague Dawley strain. All surgical procedures were done using diethyl ether for anesthesia.

Denervation was performed by removing 3–4 mm section of the deep peroneal nerve about 10 mm from its entrance into the muscle. None of the denervated muscles showed signs of reinnervation during the time course of the study Control muscles were obtained from contralateral nondenervated limbs, as well as from unoperated rats of equivalent size At 3 7 14 and 21 days after denervation the muscles were excised under continuous flow of oxygenated (95% O_2–5% CO_2) Krebs-Ringer solution having the following composition in millimoles per liter NaCl, 135 KCl, 5 $CaCl_2$, 4 $MgCl_2$, 1 Na_2HPO_4, 1 $NaHCO_3$, 15 dextrose 11 The pH of this solution was 7.2–7.3 The muscles were placed immediately into heated (30±1°C) chamber having volume of 25 ml and suffused at a rate of 4 ml/min

After an equilibration period of 20–30 min, recordings of resting membrane potentials and action potentials were made from surface fibers in the extrajunctional region which was defined as the area at least 5 mm from the end-plate zone. For these measurements, glass microelectrodes filled with 3 M KCl and having resistance of 10–15 10^6 Ω were used Action potentials were generated and recorded directly using two microelectrodes inserted in the same muscle fiber at an interelectrode distance of 50–100 µm A constant current was passed through one electrode to locally hyperpolarize the muscle fiber to −90 mV After 10–20 s an action potential was elicited by superimposing rectangular depolarizing pulse of 5 ms duration. Hyperpolarizations greater than −90 mV or

THOENEN H & TRANZER J P 1968 Chemical sympathectomy by selective de truction of adrenergic nerve endings with 6-hydroxydopamine Naunyn-Schmiedeberg s Arch Pharmakol 261 271–288

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a Physiol Scand 1980, 108: 243–46

Alterations in membrane electrical properties during long term denervation of rat skeletal muscle

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SELLIN L. C. & THESLEFF S Alterations in membrane electrical properties during long-term denervation of rat skeletal muscle. Acta Physiol Scand 1980 108, 243–46. Received 8 June 1979 ISSN 0001-6772. Department of Pharmacology University of Lund, Sweden.

Some membrane electrical properties of the extensor digitorum longus muscle of the rat were examined up to 21 days after denervation. The resting potential was significantly more depolarized at 3 days after denervation than it was at later times. The rate of rise (dV/dt) of the action potential decreased throughout the time course of the study but approached a steady value between 14 and 21 days after denervation. In addition, the dV/dt of tetrodotoxin (TTX)-resistant action potentials increased up to and including 7 days after nerve section, but declined thereafter. When expressed as % of control, the dose-response to TTX was similar throughout denervation. It is suggested that the large depolarization observed early in denervation may be related to the turnover of membrane constituents occurring at this time. The results also suggest that denervation produces reduction in the number of fast TTX-sensitive Na channels with the appearance of new population of slow TTX-resistant channels. However long term denervation results in a reduction in the density of both types of channels

Key words Denervation membrane electrical properties, sarcolemma, skeletal muscle

It is well known that denervation of mammalian skeletal muscle produces profound changes in membrane electrical properties Among these alterations are: a depolarization of the resting potential, a decrease in the rate of rise (dV/dt) of the action potential and the appearance of tetrodotoxin (TTX)-resistant action potentials (Albuquerque & Thesleff 1968 Redfern & Thesleff 1971a, 1971b) The impetus for the present study came from the observation that after an initial increase in the dV/dt of TTX-resistant action potentials early in denervation the toxin-resistance appeared to decrease between 5 and 12 days after denervation (Redfern & Thesleff 1971b). The possibility remained that the appearance of TTX-resistant Na channels could be a temporary response and may not persist during long-term denervation. Therefore, the present study was undertaken to examine the effects of long-term denervation on some of the membrane electrical properties of rat skeletal muscle

METHODS

The experiments were conducted in vitro on the extensor digitorum longus muscle (EDL) of adult male rats (250–350 g) of the Sprague-Dawley strain. All surgical procedures were done using diethyl ether for anesthesia.

Denervation was performed by removing 3–4 mm section of the deep peroneal nerve about 10 mm from its entrance into the muscle. None of the denervated muscles showed signs of reinnervation during the time course of the study Control muscles were obtained from contralateral nondenervated limbs, as well as from unoperated rats of equivalent size. At 3 7 14 and 21 days after denervation the muscles were excised under continuous flow of oxygenated (95% O_2–5% CO_2) Krebs-Ringer solution having the following composition in millimoles per liter NaCl 135 KCl 5 $CaCl_2$ 4 $MgCl_2$ 1 Na_2HPO_4 1 $NaHCO_3$ 15 dextrose 11 The pH of this solution was 7.2–7.3 The muscles were placed immediately into heated (30±1°C) chamber having volume of 25 ml and suffused at rate of 4 ml/min.

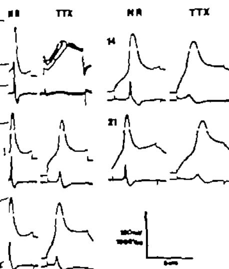
After an equilibration period of 20–30 min recordings of resting membrane potentials and action potentials were made from surface fibers in the extrajunctional region which was defined as the area at least 5 mm from the end-plate zone. For these measurements, glass microelectrodes filled with 3 M KCl and having resistance of 10–15 10^6 Ω were used Action potentials were generated and recorded directly using two microelectrodes inserted in the same muscle fiber at an interelectrode distance of 50–100 µm A constant current was passed through one electrode to locally hyperpolarize the muscle fiber to 90 mV After 10–20 an action potential was elicited by superimposing a rectangular depolarizing pulse of 5 ms duration. Hyperpolarizations greater than −90 mV or

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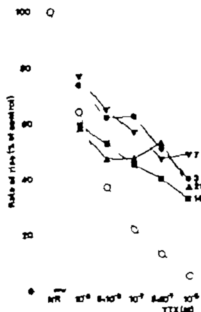


Fig. 2 Effects of various concentrations of tetrodotoxin (TTX) on the rate of rise of action potentials recorded from the extensor digitorum longus (EDL) of the rat. The open circles represent the dose–response curve for the innervated EDL, while the different days after denervation are indicated by the numbers to the right of the curves.

[...] shown previously for the extensor digitorum [...] of the rat (Albuquerque & Thesleff 1968). [How]ever the resting potential measured here at 3 [days] after denervation was substantially more de[polarized] than at later times. This was accompanied [by] the observation that larger currents were neces[sary] to hyperpolarize the membrane at three days [after] denervation than at other times. This suggests [that] applied hyperpolarizing current encountered a [sig]nificant "leak" conductance. It is difficult to [det]ermine whether this observation has particular [phy]siological significance. However it is well [kno]wn that considerable alterations occur in the [mem]brane at this time, e.g. the appearance of TTX [resi]stant Na channels and extrajunctional recep[tors], thus indicating the insertion of new membrane [comp]onents. It appears likely that this type of mem[brane] turnover may be explained by the processes [of endo]cytosis and exocytosis. High rates of endocytosis [have] been shown to begin at about 2–3 days after [de]nervation (Libelius et al. 1978, Libelius, Josefs[son] & Lundquist, 1979). It is interesting to note [th]at the process of endocytosis in the amoeba is [ac]companied by both a decrease in resting potential [an]d an increase in membrane conductance (Josefs[son], Holmer & Hansson 1975). These observations may be helpful in explaining the substantial decrease in resting potential and apparent increase in leak conductance observed at 3 days after denervation.

Tetrodotoxin had no effect on the resting potential of innervated muscles but caused significant hyperpolarization of muscles denervated for 3 and 7 days. A smaller hyperpolarization was demonstrated at 14 days after denervation but none at 21 days. This effect of TTX on denervated muscle was shown previously (Grampp, Harris & Thesleff 1972) and may be related to an abnormally high P_{Na}, which has been suggested to occur in the early stages of denervation (Robbins 1977).

The present results show that, while the rate of rise (dV/dt) of the action potential decreases steadily after denervation, the dV/dt in the presence of TTX shows a biphasic response to denervation increasing up to 7 days after denervation and decreasing thereafter. Using labelled saxitoxin (STX) Ritchie & Rogart (1977) suggested that the density of STX-sensitive channels remain unchanged after denervation and that toxin resistant action potentials reflect the presence of an entirely new popula-

Table 1 *Rate of rise (dV/dt) and overshoot of action potentials and resting membrane potential recorded from extensor digitorum longus muscles in normal Krebs Ringer solution (NR) and in Krebs Ringer with 10^{-6} M tetrodotoxin (TTX) at various days after denervation Each value indicates mean ±S*

Days after denervation	NR				TTX (10^{-6} M)			
	Fibers/muscles	dV/dt (V/s)	Overshoot (mV)	Em (mV)	Fibers/muscles	dV/dt (V/s)	Overshoot (mV)	Em (mV)
0	30/7	671±22	37 8±1.3	−78 8±0 6	28/7	–		[illegible]
3	23/6	536±29	41 4±1 7	−51 1±1 1	34/6	215±16	26 9±1.6	[illegible]
7	36/8	478±1	40.3±0 8	−57 2±1 0	48/8	232±13	33 3±1 0	[illegible]
14	50/12	381±10	36 8±0 9	−55 6±0 8	69/12	124± 9	17 9±1.3	[illegible]
21	56/13	331± 9	30 6±1 0	−56 8±0 8	61/12	122± 9	17 9±1 4	[illegible]

longer than 5 s did not increase appreciably the rate of rise of the action potential Differentiation of the action potential was accomplished by using an operational amplifier adjusted for maximum negative capacitance The time constant of the recording system was about 40 μs. These procedures were repeated after exposing the muscles to Krebs-Ringer solution containing various concentrations of tetrodotoxin (Sankyo Co Ltd Tokyo) for a period of about 20 min

RESULTS

Denervation of the extensor digitorum longus (EDL) muscle produced a fall in resting membrane potential from a control value of −78 8±0 6 mV (Mean ±1 S E) This depolarization was greatest at 3 days after denervation (−51 1±1 1 mV) At 7 (−57 2±0 8 mV) 14 (−55 6±0 8 mV) and 21 (−56 8±0 8 mV) days after denervation the resting potentials were less depolarized and similar in magnitude When 10^{-6} M tetrodotoxin (TTX) was added to the bathing solution the resting potential of the innervated muscle was unaffected (Table 1) However TTX had a marked hyperpolarizing effect on muscles which were denervated for 3 and 7 days altering them by 5 3 mV and 7 4 mV respectively This hyperpolarizing effect was less marked at 14 days (3 9 mV) and was totally absent at 1 days after denervation (Table 1)

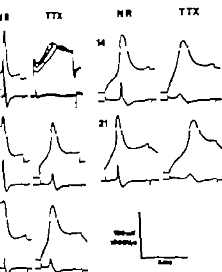
Denervation also produced alterations in some properties of directly-elicited action potentials (Table 1 and Fig 1) The rate of rise of the action potential (dV/dt) was decreased from a control value of 671±22 V/s to 536±29 V/s by the third day after nerve section At later times after denervation the dV/dt continued to decrease reaching a low value of 331±9 V/s at 21 days However the rate of decrease of dV/dt was significantly greater dur[ing] early denervation (0–3 days) than at later times [The] overshoot potential was unchanged from the co[n]trol value up to and including 14 days after dener[va]tion but was reduced at 21 days (Table 1) In ad[di]tion the repolarization rate was decreased and t[he] duration of the action potential was increased af[ter] denervation (Fig 1) Although it was not qua[nti]tated a larger current was necessary to hy[per]polarize the membrane to ~90 mV at 3 days af[ter] denervation than for innervated muscle or th[ose] denervated for longer periods

A dose–response curve for TTX was obtained [for] both the normal and the denervated muscles (F[ig] 2) TTX rapidly reduced the dV/dt of the innerv[ated] muscles at moderate concentrations and comple[tely] blocked the action potential at 10^{-6} M In contra[st] all the denervated muscles were partially resist[ant] to the action of TTX However despite the d[if]ferences in the initial dV/dt for the denerv[ated] muscles the action of increasing concentratio[ns] of TTX was similar throughout the time cou[rse] of the study

In 10^{-6} M TTX all fibers sampled generated a[c]tion potentials at 3 days after denervation Th[e] dV/dt and overshoot potential in 10^{-6} M TTX i[n]creased up to and including 7 days after nerve se[c]tion (Table 1 and Fig 1) After this time howeve[r] both the dV/dt and overshoot potential in 10^{-6} [M] TTX decreased reaching steady values betwee[n 14] and 21 days after denervation (Table 1 and Fig 1)

DISCUSSION

The fall in resting membrane potential after dener[va]vation observed in th[e present] study was similar t



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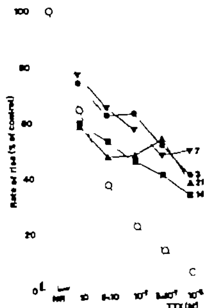


Fig. 2. Effects of various concentrations of tetrodotoxin (TTX) on the rate of rise of action potentials recorded from the extensor digitorum longus (EDL) of the rat. The open circles represent the dose-response curve for the innervated EDL, while the different days after denervation are indicated by the numbers to the right of the curves

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21	56/13	331± 9	30 6±1 0	−56 8±0.8	61/1	1[illegible]± 9	17 9±1 4	[illegible]

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Physiol Scand 1980 108. 247–252

Effects of thyroliberin and 4-aminopyridine on action potentials and prolactin release and synthesis in rat pituitary cells in culture

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SAND O HAUG E. & GAUTVIK, K. M. Effects of thyroliberin and 4-aminopyridine on action potentials and prolactin release and synthesis in rat pituitary cells in culture Acta Physiol Scand 1980 108. 247–252 Received 8 June 1979 Department of Physiology Veterinary College of Norway Oslo· Hormone and Isotope Laboratory Aker Hospital Oslo· and Institute of Physiology University of Oslo Norway

Effects of thyroliberin (TRH) and 4-aminopyridine (4AP) were studied on prolactin (PRL) secreting rat pituitary tumour cells in culture (GH cells) Intracellular recordings obtained from the same cell before and during TRH stimulation showed this peptide to increase the spontaneous firing frequency and prolong the Ca^{2+} dependent action potentials. These effects were mimicked by 4 AP which acts by interfering selectively with voltage dependent ionic channels without affecting resting membrane properties Optimal doses of TRH and 4AP approximately doubled the release of PRL. In contrast, TRH increased PRL synthesis 1.9-fold while 4AP had no effect. Increased PRL synthesis is thus not direct consequence of the hormone release. We conclude that TRH and 4AP both stimulates PRL release via the facilitating effects on the action potentials. TRH has additional intracellular effects which lead to increased synthesis of the hormone. The effects of TRH responsible for stimulation of PRL synthesis are not causally related to the impulse activity of the surface membrane of the cell

Key words Anterior pituitary action potential, thyroliberin, 4-aminopyridine prolactin release prolactin synthesis

Extracellular Ca^{2+} is a prerequisite for the stimulatory effect of thyroliberin (TRH) on prolactin (PRL) release from cultured rat pituitary tumour cells (GH cells) (Gautvik et al 1977 1979*a* Tashjian et al 1978) A certain fraction of both normal anterior pituitary cells and clonal GH cells displays an intermittent frequency of partly Ca^{2+} dependent action potentials in TRH containing medium (Kidokoro 1975 Taraskevich & Douglas 1977) Furthermore Dufy et al (1979) observed prolonged action potentials in TRH solution. By recording from the same cell before and during TRH stimulation, we have been able to confirm that TRH is responsible for these effects Both the increased firing rate and the prolongation of the action potential will increase the Ca^{2+} influx. However no direct evidence exists in favour of a functional relationship between the TRH-stimulated action potentials and the TRH-mediated hormone release

TRH could increase the resting membrane permeability to Ca^{2+} and thus induce Ca^{2+}-triggered hormone release independently of the action potentials. Such a possibility may be tested by employing drugs which exclusively affect voltage dependent permeability changes. The effects of 4-aminopyridine (4AP) on the action potentials and on the release and synthesis in the GH cells are described in the present paper This drug has no effect on resting membrane permeabilities but inhibits the late K -current in a variety of excitable cells (Pelhate & Pichon 1974 Gillespie & Hutter 1975 Llinás et al. 1976, Ulbricht & Wagner 1976 Yeh et al. 1976. Molgo et al. 1977) We find that 4AP both increases the spontaneous firing rate and prolongs the action potentials in GH cells, thus mimicking effects of TRH If TRH stimulates hormone release due to its effects on the action potentials 4AP should show a similar effect on the release. If TRH

tion of toxin resistant Na channels However it is difficult to reconcile this hypothesis with the observation that dV/dt is continually decreasing throughout denervation The present results are more in agreement with the interpretation of Colquhoun Rang & Ritchie (1974) which suggested that denervation produces a reduction in the normal fast Na^+ channels together with the appearance of slow TTX resistant Na channels Furthermore the proportion of TTX sensitive to TTX resistant channels appears to be unchanged during long term denervation The persistance of TTX resistance in long term denervation indicates that these alterations seem to be permanent and not temporary responses

We acknowledge the skilled assistance of Ms Birgitta Hansson and Ms Eva Björkbom This work was supported by postdoctoral fellowship IF32 NSO5935-01 from the National Institute of Neurological and Communicable Diseases and Stroke (NINCDS-N I H USA) and the Swedish Medical Research Council grant B76-14X 03112-06B

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Effects of thyroliberin (TRH) and 4-aminopyridine (4AP) were studied on prolactin (PRL) secreting rat pituitary tumour cells in culture (GH_4 cells). Intracellular recordings obtained from the same cell before and during TRH stimulation showed this peptide to increase the spontaneous firing frequency and prolong the Ca^{++} dependent action potentials. These effects were mimicked by 4 AP which acts by interfering selectively with voltage dependent ionic channels without affecting resting membrane properties Optimal doses of TRH and 4AP approximately doubled the release of PRL. In contrast, TRH increased PRL synthesis 1.9-fold while 4AP had no effect. Increased PRL synthesis is thus not a direct consequence of the hormone release W conclude that TRH and 4AP both stimulates PRL release via the facilitating effects on the action potentials. TRH has additional intracellular effects which lead to increased ynthesis of the hormone. The effects of TRH responsible for stimulation of PRL synthesis are not causally related to the impulse activity of the surface membrane of the cell.

Key ords. Anterior pituitary action potential, thyroliberin, 4-aminopyridine prolactin release prolactin synthesis

...racellular Ca^{2+} is a prerequisite for the stimulat... effect of thyroliberin (TRH) on prolactin (PRL) ...ase from cultured rat pituitary tumour cells (GH ...) (Gautvik et al. 1977 1979*a* Tashjian et al ...) A certain fraction of both normal anterior ...itary) cells and clonal GH cells displays an in...creased frequency of partly Ca^{2+} dependent action ...tentials in TRH containing medium (Kidokoro ...75 Taraskevich & Douglas 1977) Furthermore ...ma et al (1979) observed prolonged action po...tentials in TRH solution. By recording from the ...me cell before and during TRH stimulation, we ...ve been able to confirm that TRH is responsible ... these effects. Both the increased firing rate and ...e prolongation of the action potentials will in...crease the Ca^{2+} influx. However no direct evi...dence exists in favour of a functional relationship ...etween the TRH-stimulated action potentials and ...he TRH-mediated hormone release

TRH could increase the resting membrane permeability to Ca^{2+} and thus induce Ca^{++}-triggered hormone release independently of the action potentials Such a possibility may be tested by employing drugs which exclusively affect voltage dependent permeability changes The effects of 4-aminopyridine (4AP) on the action potentials and on the release and synthesis in the GH cells are described in the present paper This drug has no effect on resting membrane permeabilities but inhibits the late K -current in a variety of excitable cells (Pelhate & Pichon 1974 Gillespie & Hutter 1975 Llinás et al. 1976 Ulbricht & Wagner 1976 Yeh et al. 1976 Molgo et al 1977) We find that 4AP both increases the spontaneous firing rate and prolongs the action potentials in GH cells thus mimicking effects of TRH If TRH stimulates hormone release due to its effects on the action potentials, 4AP should show a similar effect on the release If TRH

tion of toxin resistant Na channels However it is difficult to reconcile this hypothesis with the observation that dV/dt is continually decreasing throughout denervation The present results are more in agreement with the interpretation of Colquhoun Rang & Ritchie (1974) which suggested that denervation produces a reduction in the normal fast Na channels together with the appearance of slow TTX resistant Na channels Furthermore the proportion of TTX sensitive to TTX resistant channels appears to be unchanged during long term denervation The persistance of TTX resistance in long term denervation indicates that these alterations seem to be permanent and not temporary responses

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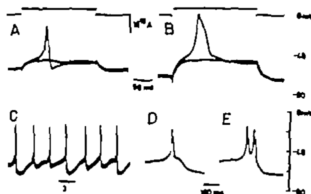


Fig. 2. Electrophysiological effects of 4-aminopyridine (4AP). (A) All or none action potential induced by depolarizing current injection in control solution. (B) Corresponding recording from different cell in solution containing 1.5×10^{-4}M 4AP. Note the retarded falling phase and absence of after-hyperpolarization. (C) Spontaneous action potentials in $5\cdot10^{-4}$M 4AP solution. Recording from the same cell at higher sweep speed showed single action potentials with pronounced shoulder on the repolarizing phase (D) or action potentials with double peaks (E).

...ted and stored PRL was therefore measured as the ...unt of hormone which accumulated in the medium ...in 20 min of incubation after addition of 4AP or TRH. ...he end of the incubation periods PRL release was terminated by transferring the tubes to ice-water and centrifuging (3 000×g) at 0°C for 5 minutes. Extracellular PRL ...measured by radioimmunoassay (Haug & Gautvik ...). All samples from one experiment were included in ...same assay series, and the determinations were performed ...duplicates. The cell protein content of the tubes ...determined by the method of Lowry et al. (1951) ...g bovine serum albumin as standard.

...RL synthesis. PRL synthesis was measured in ...onolayer cultures as the amount of hormone which accumulated in the culture medium during 48 h. The intracellular ...stores of PRL are very small, and there is no intracellular degradation of PRL (Haug et al. 1977). Furthermore ...L is stable in the culture medium under normal conditions for as long as 48 h (Tashjian et al. 1970). The extracellular amount of PRL therefore represent more than ...% of the total amount of hormone synthesized by the ...s during 48 h (Haug et al. 1977).

At the end of the treatment period the culture medium ...collected and the cells were scraped into 0.15 M NaCl ...washed 4 times. Medium and cells were stored at ...0°C until assays for PRL and cell protein were performed.

...ESULTS

...lectrophysiological registrations ...TRH effects

...uccessful impalements were characterized by ...embrane potentials and input resistances of 40–65 ...V and 500–1 500 MΩ, respectively. Action potentials could be generated in these cells either by depolarizing current injection (Fig. 2 A) or at the termination of a hyperpolarizing current pulse (Fig. 1 A). The ionic basis for the action potential and the general electrophysiological properties of the GH cell membrane have been described in detail elsewhere (Ozawa et al. 1979, Ozawa & Miyazaki 1979).

Electrically induced action potentials were recorded from 17 cells in this particular series of experiments. None of the cells fired action potentials spontaneously. Only 7 of the cells were sufficiently durable to exhibit stable membrane properties for the period required for TRH stimulation and observation of possible effects of TRH. TRH clearly altered the electrical membrane properties of 3 of these cells. Fig. 1 A shows an action potential evoked at the termination of a hyperpolarizing current pulse before TRH stimulation of one of these cells. The stimulation pipette was lowered through the solution during the time between record A and B. Record B was obtained just after the tip of the TRH pipette was positioned at a distance of 40 μm from the cell and before forced ejection of TRH had occurred. It is seen that the shape of the action potential was clearly altered even at this stage, indicating that TRH had leaked out from the pipette. The most obvious change was a prolongation of the falling phase of the action potential. TRH was then injected into the solution and after about 20 s the cell displayed spontaneous action potentials as seen in Fig. 1 C. The firing rate was about 0.7 Hz in this

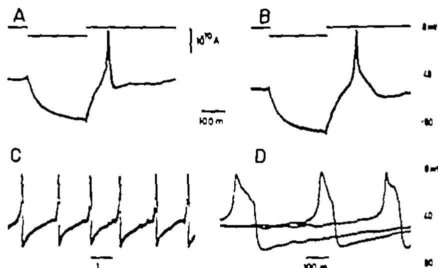


Fig 1 Electrophysiological effects of thyroliberin (TRH) (A) Action potential induced in control solution by anodal break stimulation (B) Corresponding recording immediately after positioning of the TRH pipette close to the cell Note the retarded falling phase of the action potential (C) Spontaneous activity recorded 70 s after ejection of TRH (D) Three superimposed sweeps showing the prolongation of the spontaneous action potentials relative to the electrically evoked action potentials in control solution. Same sweep speed as in A and B All recordings are from the same cell

causes release through different mechanisms 4AP would not be expected to stimulate hormone re lease The potency of 4AP to facilitate PRL release from GH_3 cells is similar to that of TRH However TRH also stimulates PRL synthesis whereas 4AP does not have this biological action

MATERIAL AND METHODS

Cell cult e The establishment and culture of the GH strain of rat pituitary tumour cells have been described previously (Tashjian et al 1968) The cell were grown as monolayers in plastic dishes containing Ham F 10 medium supplemented with 15% horse serum and .5% fetal calf serum at 37°C in a humidified atmosphere of 5% CO_2 and 95% air The medium was changed every 2–3 days Antibiotics were added to the culture med um and these did not influence cell growth o hormone synthesis (Haug & Gautvik 1976)

Electroph siological experim nt The recordings were obtained 3–10 days after subculture Just prior to an ex periment the F 10 med um wa removed and replaced with the following saline (mM). NaCl 140 KCl 5 $CaCl_2$ 10 $MgCl_2$ 1.3 glucose 10 buffered by Tris HCl 5 to pH 7 4 The experiments regarding the effect of TRH and the initial recordings in 4AP solution were performed in saline containing 70 mM CaCl (1.5 mM NaCl) since high Ca concentration is known to facilitate stable penetrations of GH cells (Kidokoro 1975). TRH was admini tered through a micropipette containing 1 μM TRH and with a tip diameter between 5 and 8 μm The t p was kept just above the surface of the solution to a oid that TRH affected the cells due to leakage from the tip during the periods between successful electrode penetrations When a stable recording was achieved from a cell displaying electrical excitabil ty the TRH pipette was slowly l ered through the solution by means of a hydraulic m drive The tip of the pipette was positioned at a dis of 30–50 μm from the cell and TRH was slowly pr injected into the solution It proved difficult to ke stable penetration for the time required for the proce The experiments invol ing 4AP were therefore perfo by comparing recordings from cells in normal sol with those obtained in saline containing between 5 and 3×10 M 4AP

The cells were viewed through an inverted micro during the experiments which were conducted at r temperature The glass microelectrodes used for in lular recording were filled from behind by capillary a with 3 M KCl or 5 M K-acetate adjusted to pH 7 acetic acid The electrode resistance ranged betwe and 250 MΩ The electrode was connected preamplifier with a facil ty for injecting current th the recording electrode using a bridge circuit (B Electronics Lab AM 1) The input resistance o preamplifier was more than 10^{12} MΩ and the input current wa les than 10^{-11} A

PRL relea e PRL release wa measured in GH_3 growing in e ther the late log rithmic or the early s nary phase of growth (6–10 days after subculture) beginning of the experiment the F 10 medium moved and the cells scraped nto Neuman-Tytell's serumless med m and washed three times. The cells then resuspended in serumles medium and aliquot of the cell suspension were added to tube or without 4AP or TRH The subsequent incubation carried out at 37°C n a shaking water bath

Previous xperiment ha e hown that when ra tive amino acid are added to the culture medium, synthesized radioacti e PRL will appear in the med min later (Gautvik & Kriz 1976) Release of alread

sition, and our data support the study by ws et al (1979) who compared recordings from rent cells exposed to various solutions The conclusions regarding the electrophysiological cts of TRH on GH_3 cells are that this peptide eases spontaneous firing and prolongs the action ntials. Our recordings show that both these ef s are clearly visible within 30 s which is the required for 2/3 of maximal binding of TRH to membrane receptors (Gautvik et al 1979*b*) It been suggested that the GH_3 cultures consist of sub-populations secreting either PRL or growth mone (Gautvik & Kriz 1976). This could explain less than half of the studied cells were clearly cted by TRH Furthermore our recordings e obtained from unsynchronized cultures and cells may have different membrane properties ending on the phase of growth

The observed electrophysiological effects of on GH_3 cells mimick certain of the effects cribed for TRH Both componds 1) increase the ntaneous firing and 2) prolong the action poten- s The action of 4AP is compatible with a reduc n of the late K -current. This will both retard the olarizing phase of the action potential and de se the after-hyperpolarization. A beginning de arization below threshold will furthermore be nced by a reduced outward-going rectification I the probability of spike initiation will thus in- ase.

The close similarity between the electrophysio- ical effects on GH cells caused by TRH and P does not necessarily reflect a common mode action for the two substances. The increased ng rate and prolonged action potentials induced TRH may for instance be caused by a direct tion on the voltage dependent Ca^{++} permeability has recently become clear that several neuro- nsmitters with presynaptic action exert their ects by interfering with the voltage depend- t Ca^{++} current in the presynaptic terminal with- t altering resting membrane properties. Pre naptic facilitation is thus associated with an in- reased Ca^{+} -permeability and prolonged action po- ntials (Klein & Kandel 1978 Pellmar & Carpenter 9), whereas reduced Ca^{++}-current and shortened tion potentials seem to be responsible for pre naptic inhibition (Dunlap & Fischbach 1978 dge et al, 1979). Klein & Kandel (1978) suggest at serotonin causes presynaptic facilitation by in- cing a cyclic AMP mediated increase of a voltage sensitive Ca^{+} current in the terminals of sensory neurons in *Aplysia* The action of TRH on the GH cell membrane might be related to such a mechanism and it has been shown that an early effect of TRH on GH cells involves cyclic AMP formation (Dannies et al 1976 Gautvik et al 1977 1979*a*).

In addition to the well established effect of 4AP on the late K -current it has recently been suggested that this drug may also have a direct effect on the voltage sensitive Ca channels in some excitable membranes (Jankowska et al 1977 Lundh & Thesleff 1977 Galindo & Rudomin 1978). However the resting membrane potential and resistance are not altered by 4AP (Pelhate & Pichon 1974 Molgo et al 1977) We therefore conclude that 4AP facilitates PRL release through a selective ef fect on the action potentials an explanation in agreement with previous reports of the effect of 4AP on neurotransmitter release (Llinás et al 1976, Jankowska et al. 1977 Kirpekar et al 1977 Lundh & Thesleff 1977 Molgo et al 1977 Galindo & Rudomin 1978)

4AP had no effect on PRL synthesis and cell growth in contrast to the marked stimulation of PRL release This stimulation was similar to that induced by TRH Furthermore 4AP which acts by selectively interfering with voltage dependent ionic channels without affecting resting membrane prop- erties mimicks certain of the effects of TRH on the action potentials. These observations support the idea that TRH enhances PRL release from GH cells via its facilitating effects on the Ca^{+} depend- ent action potentials, both regarding firing rate and spike duration Moreover since PRL secretion was stimulated by 4AP without a concomitant increase in synthesis the latter process is not necessarily a consequence of hormone release Therefore TRH regulation of PRL secretion and synthesis is prob- ably exerted through independent mechanisms as suggested by Gautvik et al. (1978)

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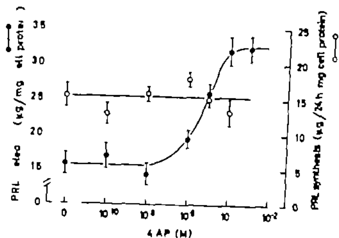


Fig 3 Effects of 4AP on PRL release and synthesis The treatment periods were 20 min for the release and ? days for the synthesis experiments Data are given as mean values ± S E of quadriplicates Values significantly dif ferent from controls ($P<0\ 05$) are marked with asterisks TRH (10^{-6}M) caused a 1 9-fold increase in PRL release and synthesis (data not shown)

cell Fig. 1 D presents 3 superimposed recordings at the same sweep speed as in records A and B and it is seen that the spontaneous action potentials were markedly prolonged compared to the electrically evoked potentials prior to TRH stimulation The remaining two cells which were visibly affected by TRH showed a similar behaviour to that presented in Fig. 1 However one of these cells was silent even after TRH stimulation although the electrical ly evoked action potentials showed a prolonged falling phase

Electrophysiological registrations of 4AP effects

Action potentials induced by current injections were recorded from 12 cells in $1-3\times10^{-3}$ M 4AP solutions In the control solution action potentials were followed by a pronounced after hyperpolariza tion (Fig 2 A) but this was abolished in the pres- ence of 4AP (Fig 2 B) Several cells in 4AP solution showed in addition a clear prolongation of the re polarization phase of the action potential as dis- played in Fig 2 B Both these alterations of the action potentials are consistent with an inhibitory effect of 4AP on the late K -current which is well developed in GH_3 cells (Ozawa et et 1979)

Spontaneous action potentials were not observed in these initial experiments which were performed in 20 mM Ca^{2+} solution High Ca^{2+} concentrations are known to reduce the excitability of cell mem- branes (Frankenhaeuser 1957) and the Ca con centration was therefore decreased to 10 mM in the remaining recordings Eleven cells were success ly penetrated in such solution containing 5×10⁻ 4AP and 4 of these cells displayed spontaneous tion potentials with a firing frequency of 0 3-1 8 Fig 2 C presents a recording from a spontane active cell and Fig. 2 D shows the shape of th the action potentials at higher sweep speed A p nounced shoulder is evident on the repolar phase of the action potential and a second act potential was occasionally initiated from this r tarded falling phase as seen in Fig. 2 E. Spontane activity was not observed in any of the 9 cells s cessfully impaled in control solution and shoul and double peaks were never seen on the electric ly induced action potentials in these cells. 10 m Ca^{2+} is an unphysiologically high concentratio and extracellular recordings from GH cells normal Ca^{2+} levels have shown the majority of ce to fire action potentials spontaneously (Kidoko 1975) However we did not achieve stable pene tions at Ca^{2+} concentrations below 10 mM

Effects of 4AP on PRL release and synthesis

Secretion of PRL was measured after 20 min incubation with 4AP in concentrations of 10^{-10} M 10 ⁻²M A dose-dependent effect was observed maximal stimulation of PRL secretion (? fold) 10 M as shown in Fig 3 It has previously be shown that 10^{-6} M TRH gives maximal stimulat of the PRL release from GH_3 cell (Dannies Tashjian 1974) and the effect of TRH at this co centration was therefore measured in the same e periment for comparison Incubation with 10^{-6} TRH for 20 min increased the PRL release from basal level of 1 6±0 15 µg/mg cell protein 3 1±0 16 µg/mg cell protein (S E. n=4) Samp were also taken already after 5 min of treatme with 10^{-4} M 4AP and 10^{-6} M TRH and a significa stimulation ($P<0\ 05$) of PRL release was observ for both compounds 4AP is thus to a large ex able to duplicate the effect of TRH on PRL releas

In contrast to the effect on PRL release 4A induced no change in the rate of hormone synthe (Fig 3) However the PRL synthesis was increa 1 9 fold in 10 M TRH solution There was no ef fect of 4AP on cell growth at any dose level

DISCUSSION

The presented intracellular effects of TRH we observed on the same l before and during TR

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Sensitization of the rat parotid gland to secretagogues following either parasympathetic denervation or sympathetic denervation or decentralization

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EKSTRÖM, J. Sensitization of the rat parotid gland to secretagogues following either parasympathetic denervation or sympathetic denervation or decentralization. Acta Physiol Scand 1980, 108: 253–261. Received 18 June 1979. ISSN 0001-6772. Department of Physiology and Biophysics, University of Lund, Sweden.

The sensitivity of the rat parotid gland to chemical agents was examined 2–3 weeks after either parasympathetic denervation or sympathetic denervation or decentralization. The parasympathetically denervated gland was markedly sensitized towards methacholine; a nonspecific supersensitivity mainly mediated via α-adrenoceptors was also demonstrated. The sympathetically denervated gland had developed supersensitivity which was both of the pre- and the postjunctional type: it was predominantly of the former type to noradrenaline and adrenaline, and entirely so to phenylephrine. It was of the latter type to isoprenaline and also to methacholine. The sympathetically decentralized gland was mainly sensitized towards noradrenaline, adrenaline and isoprenaline. The postjunctional supersensitivity developed towards the sympathomimetic drugs after sympathetic decentralization was of about the same magnitude as that observed after sympathetic denervation; this type of supersensitivity was mainly mediated via β-adrenoceptors.

Key words: Parasympathetic denervation, sympathetic denervation or decentralization, pre- and postjunctional supersensitivity, preponderance for either α- or β-adrenoceptor mediated sensitization, parotid gland, rat

Studies on the activity of the acetylcholine forming enzyme, choline acetyltransferase, and its dependence on the traffic of nerve impulses, the parotid gland of the rat has been found to be a useful model organ (see Ekström 1978). The sensitivity of the gland to chemical stimuli has in some investigations been used to indicate changes in the traffic of secretory impulses to the gland. Thus in rats kept on a liquid diet, aiming at decreased impulse traffic, the gland cells were more sensitive to the parasympathomimetic substance methacholine than those of rats kept on a dry and bulky diet (Ekström & Templeton 1977). Further loss or reduction of the traffic caused by prolonged treatment with a ganglion blocking drug sensitized the gland cells to methacholine (Ekström & Lundmark 1977, 1978). In contrast to the submaxillary gland of the rat (Ohlin 1964, Asking & Ekström 1979), few observations have been made on the effect of denervation or decentralization on the sensitivity of the parotid gland of this species to secretagogues (see Alm & Ekström 1976). In the present study the sensitivity of the parotid gland to parasympathomimetic and sympathomimetic drugs has been examined following either parasympathetic denervation or sympathetic denervation or decentralization. The parotid gland of the rat, like the submaxillary gland, belongs to those few salivary glands that have a rich sympathetic nerve supply (Norberg & Olson 1965, Alm & Ekström 1977) which on electrical stimulation causes a lively flow of saliva (Thulin 1976).

METHODS

92 adult female rats of the Sprague Dawley strain bred at the Department of Physiology were used. The animals weighed at the end of the experimental period 17±...6 g (mean ± S.E.). The operations were made under ether anaesthesia with the aid of a dissecting microscope. Parasympathetic denervation of the parotid gland was achieved by avulsion of the auriculo-temporal nerve. Sympathetic denervation by removal of the superior cervi-

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2. Threshold intravenous doses of methacholine noradrenaline adrenaline phenylephrine and isoprenaline evoking secretory responses in the parotid gland either parasympathetically denervated or sympathetically denervated or decentralized 2–3 weeks in advance and in the contralateral parotid gland

Desipramine (DMI) 1.5 mg/kg, was given intravenously previous to the test doses. Number of observations is in brackets. Values are mean ± S.E.

	Methacholine (µg/kg)	Noradrenaline (µg/kg)	Adrenaline (µg/kg)	Phenylephrine (µg/kg)	Isoprenaline (µg/kg)
Symp. den.					
Operated gland	0 14±0 03 (8)***	4.9±1.0 (7)*	4 1±0.6 (7)**	31 4±5.3 (14)*	2.7±1.0 (9)†
Contralateral gland	0 84±0 11 (8)	15.0±2.4 (7)	9.3±0.7 (7)	72.9±7.8 (14)	2.1±0 4 (9)
den.					
Operated gland	0.73±0 09 (18)**	0 65±0 08 (14)***	2.6±0.5 (11)**	16.7±2.1 (6)***	0.94±0.16 (10)
Contralateral gland	1 22±0 24 (18)	6.9 ±0 8 (14)	8.6±1.8 (11)	50 0±0 0 (6)	1.6±0.19 (10)
den. DMI					
Operated gland	Not tested	0.71±0 11 (8)*	4 7±0 3 (9)†	32.0±6 2 (10)†	Not tested
Contralateral gland		1 1±0.. (8)	5.0±0 0 (9)	32 5±6.0 (10)	
dec.					
Operated gland	1.0±0 1 (11)*	4 3±0 4 (12)**	5 8±0.6 (12)*	51 7±5 1 (12)†	0 64±0 16 (5)**
Contralateral gland	1 1±0 1 (11)	8.3±1.3 (17)	7.5±0 8 (12)	54..±4.2 (12)	1 4±0.2 (5)

†   *P*<0 05   ***P*<0 01   ***P*<0.001 when the operated gland is compared with the contralateral gland.

...and roughly it was half of that found in ...contralateral gland For the β-adrenoceptor ...ating drug isoprenaline no difference in the ...hold dose between operated and contralateral ...d was observed there was however a signifi- ...difference (p<0 01) t be found when the selec ...β-adrenoceptor stimulator H 80/6.. was tested ...rated gland 6.0±1 1 µg/kg; contralateral gland ...0 6 µg/kg; number of observations, 11). The ...unt of saliva secreted per gland in response to ...maximal doses of methacholine noradrenaline ...naline and phenylephrine was larger from the ...rated than from the contralateral gland. As to ...retion evoked by suprathreshold doses of the ...drenoceptor stimulating drugs no difference be- ...en operated and contralateral gland was found ...connection with isoprenaline whereas the results ...h H 80/6.. varied To exemplify methacholine 2 ...15 µg/kg, caused the operated gland to secrete ...±1 1 (n 8) and 14.2±1.0 (n=8) mg saliva, and ...contralateral gland 1 8±0.5 (n=8) and 4.2±0 4 ...) mg saliva. In response to noradrenaline 20 ...µg/kg, the operated gland secreted 1 9±0 6 (n=7) ...d the contralateral gland 0.5±0.2 (n=7) mg sal ... and to the same dose of adrenaline 3.5±0.8 ...=7) and 1 1 0.3 (n=7) mg saliva, respectively ...o isoprenaline 5 and 10 µg/kg, the operated gland ...creted 0 6 0 1 (n 9) and 1 4±0.. (n=9) mg sali- ...a, while the contralateral gland secreted 0.8±0.2 ...

(n=9) and 1.5±0.3 (n=9) mg saliva, in response to H 80/6.., the amount of saliva secreted at a dose of 20 µg/kg was larger (p<0 001) from the operated gland (1 6±0 2 mg saliva, =10) than from the contralateral gland (0 9±0 1 mg saliva, n=10) while at the level of 50 µg/kg there was no significant difference between the glands (operated gland. 2.6±0.5 mg saliva, contralateral gland: 1 9±0.2 mg saliva, number of observations 9) When relating the amount of saliva secreted after administration of isoprenaline to the dry weight of the gland a significantly (p<0 01) larger figure is obtained for the operated than for the contralateral gland at the dose level of 10 µg/kg, being 0 0560±0 007? (n=9) and 0 0376±0 0067 (n=9) mg saliva per mg gland The secretion evoked by isoprenaline was very viscous In some rats the injection of isoprenaline was therefore repeated after a previous injection of metacholine methacholine causing a watery secretion, was used to fill up the duct system in an attempt to reduce obstruction to the salivary flow However the amount of saliva appearing at the tip of the cannula was the same as before the injection of methacholine

Sympathetic denervation The threshold doses for all the drugs tested were lower in the operated than in the contralateral gland In the operated gland the threshold dose for methacholine was about half of that in the contralateral gland, for noradrenaline

Table I *Wet and dry weights of the parotid gland either parasympathetically denervated or sympathetically denervated or decentralized 2–3 weeks in advance and those of the contralateral parotid gland*
Number of observations is given in brackets Values are mean ± S E

	Wet weight (mg)	Operated gland/ contralateral gland (%)	Dry weight (mg)	Operated gland/ contralateral gland (%)
Parasymp den				
Operated gland (18)	85 6±3 4	59 6±2 2	4 6±1 0	59 9±2 5***
Contralateral gland (18)	146 –±7 1		4– ±–	
Symp den				
Operated gland (50)	1?0 7±3.2	87 4±2.1	3 .3±1 0	86 1± 7***
Contralateral gland (50)	139.3±3 3		38 1±1 1	
Symp dec				
Operated gland (4)	116 7±4 6	89 8±2 7*	33 0±1 –	93 4±3 5*
Contralateral gland (24)	131 –±5 4		36 1±1 6	

$P<0.05$ $P<0.001$ when the operated gland is compared with the contralateral gland.

cal ganglion and sympathetic decentralization by cutting the cervical sympathetic nerve trunk The surgical procedure was made unilaterally the contralateral gland served as control 2–3 weeks after surgery the animals were anaesthetized with chloralose (100 mg/kg) through a femoral venous cannula after induction with ether The animals were provided with a tracheal cannula and the body-temperature was checked with a rectal thermometer On both sides the duct of the parotid gland was exposed near its entrance in the mouth and cannulated using a fine glass cannula, which gave about 115 drops from 1 ml distilled water Saliva appearing at the tip of the cannula was collected on filter paper and weighed The amount of saliva secreted was expressed in mg saliva per gland or in mg saliva per mg dry weight of the gland To estimate threshold dose and the submaximal secretory responses a series of standard doses of methacholine chloride (usually in the range of 0 01–10 μg/kg) noradrenaline bitartrate (0 1–20 μg/kg) adrenaline bitartrate (0.5–?0 μg/kg) phenylephrine hydrochloride (1 100 μg/kg) and isoprenaline sulphate (0.5–10 μg/kg) were injected through the femoral cannula In some experiments the selective β-adrenoceptor stimulating drug H 80/6– was also used (see Ekström 1979) since the drug has a long duration of action the amount of saliva secreted was expressed per 5 min Because of deterioration of the preparation the number of drugs tested on each rat was usually restricted to two or three After the injection of the largest dose of the sympathomimetic drugs a respiratory arrest was often observed except for isoprenaline and H 80/6– In such a case artificial respiration was temporarily given by blowing air into the tracheal cannula. When desmethylimipramine hydrochloride 1.5 mg/kg was used to inactivate the neuronal amine pump the drug was injected slowly i.v over a period of 10 min 10 min thereafter the first of the standard doses was injected

At the end of the acute experiment the parotid gland were removed cleaned pressed between gauze pads and weighed before (wet weight) and after heating to 105–110°C for 48 h (dry weight).

Student's *t* test was used paired comparisons were made between the operated gland and the contralateral unoperated gland The 0 05 level of probability was considered significant

RESULTS

Gland weights

After parasympathetic denervation both the wet and the dry weight of the operated gland was found to be about 60% of the contralateral gland (Table 1) The wet and the dry weight of the sympathetically denervated gland was 87 and 86% of the contralateral gland respectively the corresponding percentage figures for the sympathetically decentralized gland were 90 and 93 (Table 1) There was no significant difference between the weights (wet or dry) of the sympathetically denervated gland and those of the sympathetically decentralized gland when comparisons were made on percentage basis (operated gland as percent of contralateral gland)

Secretory responses

Threshold doses for the drugs are shown in Table and the amounts of saliva secreted in response to submaximal doses in Figs 1–4

Parasympathetic denervation In the operated gland the threshold dose for methacholine was one-sixth for noradrenaline one-third and for adrenaline half of that needed to evoke secretion in the contralateral gland The threshold dose for the α-adrenoceptor stimulating drug phenylephrine was also lower in the the contral

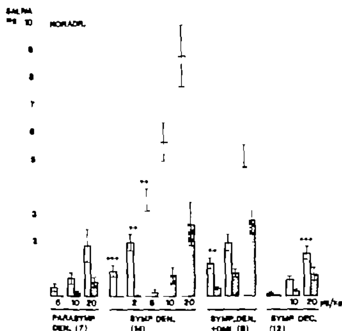


Fig 2 Noradrenaline. Secretory responses, in mg saliva, of various intravenous doses, in µg/kg, of noradrenaline from parotid glands unoperated (hatched columns) or either parasympathetically denervated or sympathetically denervated or decentralized, 3 weeks in advance (open columns). DMI indicates the presence of desmethylimipramine. For further explanations see Fig. 1

...ated gland secreted larger amount of saliva ... the contralateral gland although the differ... were not at all as marked as in the group of ... where these drugs were tested without the pres... of desmethylimipramine. For instance nor... ...naline, 2 and 5 µg/kg, evoked a secretion of ...±0.3 (n=8) and 4.8±0.8 (n=8) mg saliva from ... operated gland and from the contralateral gland ...0 (n=8) and 2.8±0.5 (n=8) mg of saliva, and ...naline, 5 and 10 µg/kg, evoked a secretion of ...±0.3 (n=9) and 2.1±0.2 (n=9) mg saliva from ... operated gland, and 1.0±0.1 (n=9) and 1.7±0... ...) mg saliva from the contralateral gland. In ... group of rats neither noradrenaline nor ad...naline was tested at the dose level of 20 µg/kg. In ...ponse to phenylephrine 50 and 100 µg/kg, the ...ount of saliva secreted from the operated gland ... not differ significantly from that secreted from ... contralateral gland, neither were there any dif...ences to be found were the comparisons between ...erated and contralateral gland to be based on mg ...liva secreted per mg dry tissue weight. In the rats ...eated with desmethylimipramine the contralateral glands secreted larger amount of saliva than the corresponding glands in the rats not treated with this drug, as can be seen in the Figs. ...–4.

Sympathetic decentralization. The threshold dose for methacholine in the separated gland did not differ significantly from that in the contralateral gland, neither was there any difference to be found for phenylephrine. For noradrenaline and isoprenaline the threshold dose in the operated gland was about half of that in the contralateral gland. For adrenaline the threshold dose was also lower in the operated than in the contralateral gland, although the difference in threshold dose between the glands was not as big as that found for noradrenaline and isoprenaline. In response to submaximal doses of methacholine and phenylephrine a difference between operated and contralateral gland could only be demonstrated at some dose levels provided the amount of saliva secreted was related to the tissue weight. Thus at 5 and 10 µg/kg of methacholine the operated gland secreted 0.1364±0.0139 ($n=11$) and 0.2683±0.0213 ($n=11$) mg saliva per mg gland, while the contralateral gland secreted 0.1099±

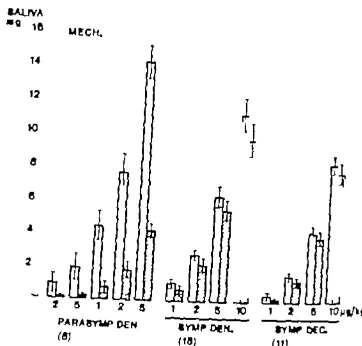


Fig 1 Methacholine Secretory responses in mg saliva of various intravenous doses, in µg/kg, of methacholine from parotid glands unoperated (hatched columns) or either parasympathetically denervated or sympathetically denervated or decentralized 3 weeks in advance (open columns) The column and its vertical bar is mean ± S E. Number of observations is indicated in brackets. Comparison was made between the operated gland and its contralateral unoperated gland in the case of a significant difference this is shown as follows $P<0\ 05$ $P<0\ 01$ $P<0\ 001$

one-tenth for adrenaline and phenylephrine about one third and for isoprenaline roughly half of that in the contralateral gland To all the drugs the secretion in response to submaximal doses was larger from the operated than from the contralateral gland although to methacholine the difference was not very conspicuous The amount of saliva secreted in response to methacholine 2 and 5 µg/kg was from the operated gland 2 8±0 3 ($n=18$) and 6 3±0 6 ($n=18$) mg saliva and from the contralateral gland 2 2±0 4 ($n=18$) and 5 5±0 6 ($n=18$) mg saliva Of the sympathomimetic drugs the most marked differences were observed in connection with noradenaline adrenaline and phenylephrine e g noradrenaline 10 and 20 µg/kg, evoked a secretion of 5 6±0 7 ($n=14$) and 8 7±1 1 ($n=14$) mg saliva from the operated gland and 0 8±0 3 ($n=14$) and 2 6±0 8 ($n=14$) mg saliva from the contralateral gland adrenaline 10 and 20 µg/kg 2 6±0 5 ($n=11$) and 5 1±0 8 ($n=11$) mg saliva from the operated gland and 0 4±0 2 ($n=11$) and 1 9±0 4 ($n=11$) mg saliva from the contralateral gland and finally phenylephrine 50 and 100 µg/kg 3 8±0 3 ($n=6$) and 10 9±1 1 ($n=6$) mg saliva from the operated gland and 0 2±0 1 ($n=6$) and 2.4±0 6 ($n=6$) mg saliva from the contralateral gland As to isoprenaline the amount of saliva secreted was at a dose level of 5 µg/kg ? 1±0.2 ($n=10$) from the operated gland and 1.2±0.3 ($n=10$) mg saliva from the contralateral gland and at a dose level of [illegible] µg/kg 3 1±0 3 ($n=10$) and 1 9±0 4 ($n=10$) mg saliva, respectively

Sympathetic denervation + desmethylimipramine In those rats where the neuronal amine pump was inactivated previously to the administration of noradrenaline adrenaline and phenylephrine a lower threshold dose in the operated than in the contralateral gland could only be demonstrated for noradrenaline however the difference in threshold between operated and contralateral gland was small the dose needed to evoke secretion in the operated gland was not even half of that needed to evoke secretion in the contralateral gland. When comparing the threshold doses of the contralateral gland in the desmethylimipramine treated rats with those of the contralateral glands in rats not treated with this drug it can be seen from Table that the threshold doses in the treated rats are lower than those in the untreated rats In response to submaximal doses of noradr and adrenaline the

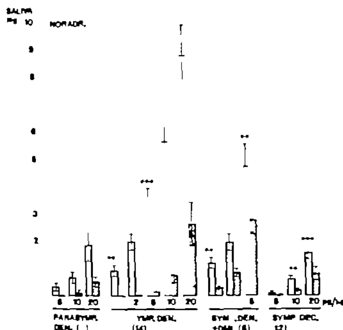


Fig 2 Noradrenaline. Secretory responses, in mg saliva, of various intravenous doses, in µg/kg, of noradrenaline from parotid glands, unoperated (hatched columns) or either parasympathetically denervated or sympathetically denervated or decentralized, 3 weeks in advance (open columns); DMI indicates the presence of desmethylimipramine. For further explanations see Fig. 1

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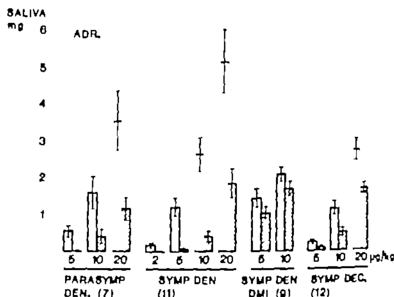


Fig 3 Adrenaline Secretory responses in mg saliva of various intravenous doses, in µg/kg of noradrenaline from parotid glands unoperated (hatched columns) or either parasympathetically denervated or sympathetically denervated or decentralized 3 weeks in advance (open columns) DMI indicates the presence of desmethylimipramine For further explanations see Fig 1

0 0105 ($n=11$) and 0 ?297±0 0193 ($n=11$) mg saliva per mg gland respectively the difference was significant at a p level of <0 05 At 100 µg/kg of phenylephrine the operated gland secreted 0 1?43± 0 0?09 ($n=12$) and the contralateral gland 0 0904± 0 01?9 ($n=12$) mg saliva per mg gland (p<0 0?) With regard to submaximal doses of noradrenaline adrenaline and isoprenaline the amount of s[...] secreted in response to these drugs was larger f[...] the operated than from the contralateral gland [...] instance the operated gland secreted 0.[...] ($n=1$?) and the contralateral gland 0.2±0.1 ([...] mg saliva after the injection of noradrenaline [...] µg/kg and after ?0 µg/kg of this drug 1[...]

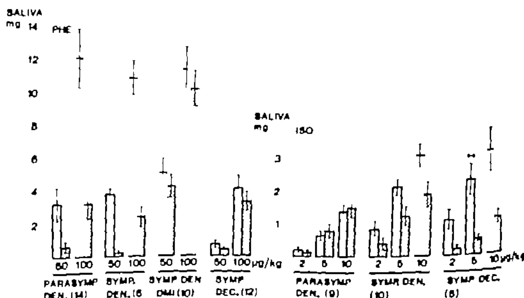


Fig 4 Phenylephrine to the left and isoprenaline to the right Secretory responses in mg saliva of various intra[...] doses in µg/kg, of phenylephrine and of isoprenaline from parotid gland unoperated (hatched columns) or parasympathetically denervated or sympathetically denervated or decentralized 3 weeks in advance (open col[...] DMI indicates the presence of desmethylimipramine For further explanation see Fig 1

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nces ere obtained in response to isoprena-
line in response to 5 and 10 μg/kg of the drug,
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0.5±0.1 (=5) and 1 1±0 2 (n=5) mg saliva,
tively

USSION

arotid gland cells were found to be sensitized
eeks postoperatively using the threshold dose
cretion and the amount of saliva secreted in
nse to supramaximal doses of the drugs as
a for supersensitivity (see Emmelin 1952),
type of operation giving rise to characteristic
res

ulsion of the auriculo-temporal nerve in the rat
s an almost complete disappearance of the
nergic nerves within the glands judged by the
ked reduction in the activity of choline acetyl-
ferase (Ekstrom 1974) and in the number of
nesterase positive nerves (Alm & Ekstrom
). The number of adrenergic nerves does not
to be reduced by avulsion of the nerve (Alm &
röm 1977). As would be expected such a
sympathetically denervated gland showed a
ed sensitization towards methacholine in a
ious study on the rat parotid gland (Alm &
röm 1976) here the secretory responses to
threshold doses of methacholine were tested
persensitivity to this drug was demonstrated
wing parasympathetic denervation. Supersen-
ity is normally considered an unspecific phe
enon (Cannon & Rosenblueth 1949) and in
present study sensitization to some of the sym-
homimetic drugs as found In the rat parotid
nd the presence of α- and β-adrenoceptors both
ducing secretion of fluid has previously been
onstrated (Ekstrom 1973). In the present inves-
tion the observation was made that following
sympathetic denervation it was particularly the
drenoceptors of the gland that were sensitized
spite of the marked glandular atrophy the secre
ry responses to isoprenaline were not even reg
arly increased in the denervated gland when the

amount of saliva produced was related to the tissue weight the results obtained in connection with H 80/62, the β-selective adrenoceptor stimulating drug did also vary

Removal of the superior cervical ganglion causes most of the adrenergic nerves in the rat parotid gland to degenerate (Alm & Ekström 1977); such a denervation does not seem to affect the cholinergic nerves of the gland as judged both from studies using histochemistry (Alm & Ekström 1976) and from those determining the activity of choline acetyltransferase (Ekström 1977). In the sympathetically denervated parotid gland the sensitization developed was particularly marked to noradrenaline adrenaline and phenylephrine It was less marked to isoprenaline and even less to methacholine In the presence of desmethylimipramine the contralateral gland became sensitized which had the consequence that the difference in the response to noradrenaline and adrenaline between the sympathetically denervated gland and this gland was diminished and to phenylephrine a significant difference could no longer be demonstrated. Isoprenaline was not tested in animals given desmethylimipramine since it is not considered to be taken up by the neuronal amine pump (Iversen 1967)

The sensitization de eloped in the sympathetically decentralized gland to noradrenaline and adrenaline was below that attained after sympathetic denervation this was however not the case for isoprenaline As to phenylephrine and also to methacholine increased secretory responses from the decentralized gland could only be obtained at some dose levels provided the responses were expressed per tissue weight It may thus be concluded that sympathetic denervation of the rat parotid gland is followd by a sensitization that is both of pre and postjunctional type the latter type not only indicated by the upersensitivity to isoprenaline but also by the unspecific supersensitivity to methacholine. It was predominantly of the prejunctional type to noradrenaline and adrenaline and probably entirely so to phenylephrine as shown by the use of desmethylimipramine Further the postjunctional supersensitivity developed towards the sympathomimetic drugs seemed particularly to be mediated via β-adrenoceptors, as seemed to be the case also for the postjunctional supersensitivity developed after the ympathetic decentralization of the gland

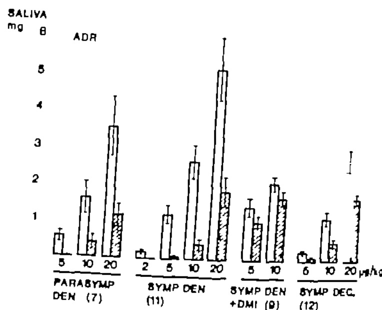


Fig 3 Adrenaline Secretory responses in mg saliva, of various intravenous doses, in μg/kg of noradrenaline from parotid glands unoperated (hatched columns) or either parasympathetically denervated or sympathetically denervated or decentralized 2 3 weeks in advance (open columns) DMI indicates the presence of desmethylimipramine For further explanations see Fig 1

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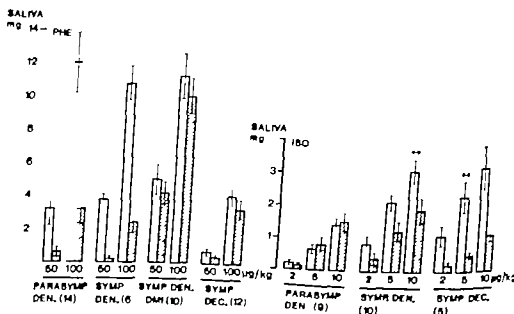


Fig 4 Phenylephrine to the left and isoprenaline to the right Secr tory responses in mg sali a of various intraven. doses, in μg/kg, of phenylephrine and of isoprenaline from parotid gland unoperated (hatched columns) or either parasympathetically denervated or sympathetically denervated or decentralized 3 weeks in advance (open columns) DMI indicates the presence of desmethylimipramine For further explanations see Fig. 1

reponderance for an α-mediated sensitization rat parotid gland following parasympathetic ation.

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The finding that the sympathetically decentralized parotid gland lacked obvious signs of sensitization to methacholine makes it likely that the supersensitivity found towards this drug in rats given a liquid diet (Ekström & Templeton 1977) and in those treated with a ganglion blocking drug (Ekström & Lindmark 1978) was primarily due to reduction or abolishment of the traffic of secretory impulses in the postganglionic parasympathetic nerves of the parotid gland and not to changes in the postganglionic sympathetic nerves of the gland

Many studies have been devoted to the effect of parasympathetic denervation and decentralization on the sensitivity of salivary glands (see Emmelin 1965) Only few studies have dealt with the effect of sympathetic denervation and decentralization one reason for this is probably the considerable variation in the sympathetic secretory innervation of salivary glands However in the cat s submaxillary gland Emmelin & Engström (1960) found after sympathetic denervation supersensitivity to noradrenaline and adrenaline but not regularly to parasympathomimetic drugs Sympathetic decentralization of this gland caused no supersensitivity which suggested to these authors that the traffic of secretory impulses in the adrenergic nerves of the gland was low In the rat both submaxillary and parotid glands are supplied with sympathetic nerves that take part in digestive reflexes causing secretion both of fluid and of organic material (Ohlin 1968 Garrett & Harrop 1976 Speirs & Hodgson 1976) Ohlin (1968) found the sympathetically decentralized submaxillary gland to weigh slightly less than its contralateral gland and further it seemed to have a lower threshold dose for noradrenaline than its control In the present study the sympathetically decentralized parotid glands were also found to weigh less than their contralateral glands and it was possible to demonstrate significant differences in the sensitivity to sympathomimetic drugs between decentralized and control glands

When comparing the secretory responses towards noradrenaline adrenaline and isoprenaline from the sympathetically denervated gland in the case of noradrenaline and adrenaline in the presence of desmethylimipramine with those from the sympathetically decentralized gland a striking feature seems to be that the degree of postjunctional sensitization developed after denervation appears to be similar to that after decentralization A similar relationship between sympathetically denervated and sympathetically decentralized parotid [illegible] the rat was recently found in connection [illegible] sensitization that was demonstrated towards [illegible] selective β-adrenoceptor stimulating drug [illegible] (Ekström 1979) Parasympathetic denervation [illegible] salivary glands is known to cause a more pronounced supersensitivity than parasympathetic decentralization and this is explained by the fact [illegible] the denervation does not only exclude the [illegible] the gland cells of that fraction of the transmitter [illegible] is released by the arrival of secretory impulses [illegible] also the action on the gland cells of that fraction [illegible] the transmitter that is continuously released from the nerve endings (see Emmelin 1965). The [illegible] of the present study combined with those of [illegible] ström (1979) may suggest the possibility that [illegible] continuous release of the adrenergic transmitter [illegible] of minor importance for the level of sensitivity [illegible] the parotid gland cells this may depend on [illegible] ficient amount of the transmitter acting on the [illegible] perhaps due to too a small continuous release [illegible] the transmitter from the nerve endings or [illegible] effective amine uptake mechanism at the [illegible] certain continuous release of the adrenergic transmitter in analogy with that of the choline[illegible] transmitter has previously been discussed [illegible] Emmelin (1967) the fact that in the present investigation unambiguous signs of supersensitivity [illegible] wards methacholine was found after sympathetic denervation but not after sympathetic decentralization may perhaps be taken as a support for [illegible] possibility

In salivary glands parasympathetic and [illegible] pathetic nerves are considered to act on the [illegible] secretory cell (see Emmelin 1967) [illegible] was mainly the α-adrenoceptors that in the present study seemed to mediate sensitization to [illegible] pathomimetics following parasympathetic denervation This may imply that in the rat parotid [illegible] not all cells supplied with β-adrenoceptors are [illegible] der the influence of the parasympathetic [illegible] On the other hand it has been suggested from [illegible] vitro studies on slices on rat parotid tissue that [illegible] same cell is supplied both with α- and β adreno[illegible] tors and with muscarinic cholinergic receptors [illegible] further that α-adrenoceptors and cholinergic receptors use the same intracellular mechanism [illegible] β-adrenoceptors use another one (Schramm & [illegible] linger 1975 Leslie Putney Jr & Sherman [illegible] is tempting to hypothesize that such an intra[illegible] arrangement may be th [illegible] ation for the [illegible]

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aerobic threshold skeletal muscle enzymes d fiber composition in young female ss-country skiers

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RUSKO H RAHKILA P & KARVINEN E.. Anaerobic threshold skeletal muscle enzymes and fiber composition in young female cross-country skiers. Acta Physiol Scand 1980 108.263–268 Received 19 June 1979 ISSN 0001-6772. Department of Biology of Physical Activity University of Jyvaskyla, Finland.

Anaerobic threshold (AT) and maximum oxygen uptake (max V_{O_2}) were determined in 15 young female cross-country skiers aged 15–20 years, during incremental bicycle ergometer exercise Succinate dehydrogenase (SDH) malate dehydrogenase (MDH), citrate synthase (CS) and lactate dehydrogenase (LDH) were analyzed biochemically and percentage of slow twitch fibres (%ST fibres, myosin adenosine triphosphatase staining) histochemically in muscle samples obtained from m. vastus lateralis. Max V_{O_2} correlated significantly with anaerobic threshold in ml kg min (mlAT) but when AT was expressed in percent of max V_{O_2} (%AT) the correlation was insignificant Significant correlations were found between %AT and SDH (r=0 63) and between mlAT and CS (r=0 58) Max V_{O_2} showed no significant correlations with the enzymes studied or %ST fibres The results of the study seem to support the hypothesis that anaerobic threshold is related to oxidative capacity of muscle.

Key words Anaerobic threshold maximum oxygen uptake, enzyme activities, muscle fiber composition, athletes

I ell established that endurance training will use significant functional and structural adaptations both in the cardiovascular system and in letal muscle Maximum oxygen uptake (max) is thought to reflect these adaptations because it has been shown to correlate with heart lume cardiac output muscle capillarization scle fiber composition, mitochondrial density tivities of oxidative enzymes etc. (see Holloszy Booth 1976 Saltin 1977 Åstrand & Rodahl 1977) Max V_{O_2} might be the most important determinant of endurance performance when large scle mass is activated during maximum exercise of short duration During prolonged exercise lasting 3 h) 60-90% of max V_{O_2} can be used has been suggested that the endurance of hletes can be characterized physiologically in lation to max V_{O_2} as the highest intensity of ercise at which the production of lactic acid is ot increased (Wyndham 1969 Costill et al 1973) Incremental bicycle ergometer tests have been developed to estimate this intensity of exercise that is termed the anaerobic threshold (AT) AT may be detected from the increase in blood lactate concentration above normal resting levels and the non-linear increase in minute ventilation and carbon dioxide production (Wasserman et al 1973 Davis et al 1976 Mader et al 1976 Bachl et al 1978)

The purpose of this study was to determine the anaerobic threshold of young female cross-country skiers and to investigate the relationships between anaerobic threshold maximum oxygen uptake and oxidative capacity of skeletal muscle

MATERIAL AND METHODS

The subjects were 15 female cross-country skiers, aged 15 to 20 years. They were among the best girl skiers in Finland in their age groups and had trained (running

We deeply regret that our honoured and dearly beloved teacher Professor Esko Karvinen, Ph D died before the publication of this paper

2. V̇o₂ maximum and anaerobic threshold—mean values of the 15 subject

Max V_{O_2} (l × min⁻¹)	STPD (ml × kg⁻¹ min⁻¹)	Max heart rate	Max blood lactate	Anaerobic threshold (ml kg⁻¹ min⁻¹)	% max V_{O_2}	Heart rate
[illegible]	47.3	193.5	8.2	40.9	85.7	180.4
0.28	3.6	8.1	1.4	3.3	6.6	9.2

[illegible] mean value of %ST fibres and muscle [illegible]ties of SDH, MDH, CS, and LDH are pre[illegible]d in Table 3. On the average the girl skiers [illegible]0% ST fibres in their m. vastus lateralis. The [illegible] fibres showed no significant correlation with [illegible] mlAT or max V[illegible] SDH correlated signifi[illegible] [illegible]ith %AT (=0.63, $P<0.05$) and CS with [illegible] (0.58, $P<0.05$). Sum of the z-scores of the [illegible]ative enzymes (ΣOE) correlated significantly [illegible] %AT ($r=0.54$, $P<0.05$). The correlations be[illegible] max V_{O_2} and the enzymes studied were [illegible]ficant. LDH correlated significantly with [illegible]ty of training ($r=-0.51$, $P<0.05$) and with [illegible] the subjects ($r=-0.51$, $P<0.05$).

[illegible]USSION

[illegible] concept of anaerobic threshold has been [illegible]ed to describe the intensity of exercise or [illegible] consumption at which a person can work [illegible]rolonged time. It may be detected from the [illegible] in blood lactate concentration and as[illegible]ed changes in respiratory parameters during [illegible]mental exercise (Wasserman et al. 1973). [illegible] changes have been thought to reflect the [illegible] of metabolic acidosis and to show that the [illegible] of the aerobic energy production is no more [illegible] to fulfill the requirements of exercise. In our [illegible] procedure the intensity of exercise was [illegible]sed every second minute by 15 W corre[illegible]ing to an increase of 0.15–0.20 l × min⁻¹ in [illegible] It was supposed that the individual AT values [illegible] also be between two successive work loads. [illegible]fore it was tried to determine the AT at the [illegible]acy of 5 W or 0.05–0.10 l × min⁻¹ (1 ml × [illegible] min⁻¹). This corresponds to an increase of [illegible] in %AT. In some cases %AT was surpris[illegible] high. For instance, subject 2 attained an [illegible] value of 45 ml × kg⁻¹ × min⁻¹ which is 3rd [illegible] in the group. After that intensity of exercise [illegible]V_{O_2} levelled off and blood lactate concentration increased from 4.1 mM to 8.2 mM. Apparently she was not able to increase her $\dot V_{O_2}$ after she had attained the AT. Consequently her max V_{O_2} was relatively low and %AT value clearly highest in the group. Four months later the %AT of this girl was still highest in the group.

In our study a 4 mM (Mader et al. 1976) threshold value of blood lactate concentration was applied. It has been shown that after marathon race the blood lactate concentration of runners is 1.1–3.5 mM (Costill & Fox 1969) and according to Costill et al. (1973) athletes are able to work for prolonged time at blood lactate level of 4 mM or lower. If the intensity of exercise is higher the blood lactate concentration increases all the time. In our study some of the subjects attained high blood lactate values already at low intensity of exercise. For instance, subject 12 had blood lactate concentration of 4.3 and 4.8 mM after the 5th and 7th work loads, respectively (see Fig. 1). The beginning intensity of exercise and the first 30 W increments might have been too high in relation to the working time of two minutes at each intensity of exercise. An oxygen deficit could have been developed and as a result lactate production starts to increase. On the other hand, some of the subjects had blood lactate values below 2 mM at low intensity of exercise indicating that oxygen deficit had not been high in the beginning of the test. For instance

Table 3. Muscle fiber distribution and activities of th[illegible] enzym[illegible]s studied

n=15	% ST fibres	Enzyme activities, (nmol · min⁻¹ × mg⁻¹ prot) SDH	MDH	CS	LDH
$\bar x$	59.9	13.9	1 776	144	839
S.D.	9.6	2.8	469	41	182

Table 1 *Characteristics and training data for all subjects*

$n=15$	Age (y)	Height (cm)	Weight (kg)	Body fat[a] (%)	Quantity of training during the preceding year[b] (km × year^{-1})
$\bar{x}$	17 6	166 9	57 7	22.7	3 330
S D	1 4	5 1	5 1	2 3	670

[a] Skinfold estimation according to Durnin & Rahaman (1967)
[b] Obtained from the training diaries of the subjects

walking skiing rollskiing) on the average 3 300 km during the preceeding year Information on their training and their physical characteristics are seen in Table 1 The subjects gave their informed consent to participate in the measurements of this study

AT and max V_{O_2} were determined during performance on a bicycle ergometer (60 rpm) The beginning intensity of exercise was 90 W and the power was increased every second minute by 30 W during the first 2–4 increments (according to the fitness level of the subject) and thereafter by 15 W every second minute until exhaustion which occurred after about 20–25 min of exercise

During the test ventilation (V_E) oxygen uptake (V_{O_2}) and carbon dioxide production (V_{CO_2}) were measured using a semiautomated system The subject breathed through a modified Otis McKerrow respiratory valve and the expirations were directed into a smooth tube (Ø 30 mm) The expiratory volume was measured using a heated Fleisch 3 pneumotachograph and a Penyves & Gut flow meter This system was calibrated before and after the tests by pumping 100 l of air through the pneumotachograph using a 4 l cylinder Samples from every expiration were collected in relation to flow into a sample bag. For every 30 s period ventilation was recorded and O_2- and CO_2-concentrations were analyzed from a sample bag calibration gas (analyzed by Scholander technique) and room air by Beckman OM 11 and Godart Capnograph respectively The temperature of the expired air was measured in the pneumotachograph and the respiratory volumes (V_E V_{CO_2} V_{O_2}) were calculated for every 30 s period and corrected for temperature and ambient pressure. The highest sum of two successive 30 s determinations was taken as maximum oxygen uptake

EKG for heart rate calculations was recorded at the end of every minute Blood samples for lactate determination (enzymatic method Biochemica Boehringer) were drawn from fingertip after the 5th and the 7th work load and again immediately after and 2.5 min after the test

To determine AT V_E and V_{CO_2} were plotted against corresponding V_{O_2} and heart rate values Fig. 1 Departure from linearity in the V_E- and V_{CO_2}-responses was used as a criterion of anaerobic threshold The increase of blood lactate concentration over 4 mM was used together with the respiratory responses to verify that AT had been reached AT was determined by hand and calculated in ml O_2 consumed × kg^{-1} × min^{-1} (mlAT) and in percent of maximum oxygen uptake (%AT) Correlation between the determinations of two investigators was 0 9 The reliability of the testing procedure was studied separately on 12 physical education students. The correlation between their test–retest (one week between the tests) values were 0 98 (l × min^{-1}) and 0 94 (ml × kg^{-1} × min^{-1}) for max V_{O_2} and 0.95 (l × min^{-1}), 0.9[illegible] (ml × kg^{-1} × min^{-1}) and 0.80 (% of max V_{O_2}) for AT

A muscle sample from m vastus lateralis of left [illegible] was obtained using needle biopsy technique (Bergström 1962) The specimen was divided into two parts One portion (10–15 mg) for histochemical analysis was mounted [illegible] OCT compound and frozen in isopentane cooled [illegible] liquid nitrogen The sample was sectioned in a cryostat and stained for myosin ATPase (Padykula & Herman 1955) Microphotographic enlargements of the slides [illegible] used to classify the muscle fibres to slow twitch [illegible] and fast twitch (FT) types (Gollnick et al 1972) The other portion (5–15 mg) of the muscle sample was frozen [illegible] liquid nitrogen and stored at −80°C for subsequent enzyme analysis. After smelting on ice-bath these [illegible] pieces were weighed and homogenized in [illegible] 1 M Tris buffer pH adjusted to 7.5 in an all glass [illegible] Potter Elvehjelm homogenizer operated [illegible] bath The following enzymatic activities were [illegible] from the original homogenate or after appropriate [illegible] tions succinate dehydrogenase (SDH E.C [illegible] according to Pennington (1961) citrate synthase (CS E [illegible] 4 1 3 7) according to Srere (1969) malate dehydrogenase (MDH E C 1 1 1 37) according to Ochoa ([illegible] lactate dehydrogenase (LDH E.C 1 1 1.27) according [illegible] Kornberg (1955) All preparation steps were performed [illegible] 0–4°C The specific enzyme activities at 22°C were [illegible] ferred to protein content estimated according to Lowry et al (1951) To estimate the total oxidative capacity [illegible] the muscle the sum of the z-scores of the three oxidative enzymes (ΣOE) was calculated

RESULTS

Mean max V_{O_2} of the subjects was 2 69 l × min^{-1} or 47 3 ml × kg^{-1} × min^{-1} The AT averaged 40 9 ml × kg^{-1} × min^{-1} which was 86% of max V_{O_2} (Table 2) Maximal oxygen uptake (ml × kg^{-1} × min^{-1}) correlated positively ($r=0$ 60 $P<0$[illegible]) with mlAT but correlation between max V_{O_2} and %AT was insignificant The age of the subjects correlated positively with %AT ($r=0.54$ $P<0$[illegible]) and negatively with max [illegible] 63 $P<0$ 05[illegible]

es and blood and (3) oxygen transport to king muscles. In our study high quantity of ng seemed to be related to low LDH activity igh anaerobic threshold. Although the nega- correlation between LDH and %AT (r=) as insignificant, these results seem to show lency that the ability to keep the blood lactate ntration at low level might also be related to pacity of the muscle to produce lactate.

highest measured max V_{O_2} values have been ed from cross-country skiers (Åstrand & hl 1977). In our study the mean max V_{O_2} 0 ml × kg × min⁻¹. Higher values for female rs of the same age and for cross-country s have been reported (Costill et al. 1976 nd & Rodahl 1977 Rusko et al. 1978). To extent the low values observed in this study be explained by methodological differences ng bicycle ergometer exercise the max V_{O_2} out 7% lower than during treadmill running. r study the duration of bicycle ergometer test 20-25 min which is not optimal for attaining max V_{O_2} (Åstrand & Rodahl 1977). The tests performed 3 months after the main competi- and according to Simon et al. (1978) cross- try skiers have lowest max V_{O_2} values at the of the competition season.

e results of the study allow to present a com- t on the effect of training in young female cross- try skiers. The significant correlation between and training (0.70, $P<0.01$) indicates that s the skiers become older they train quanti- ely more. The age of the subjects correlated tively with %AT while negative correlation be- en age and max V_{O_2} was observed. Accordingly increased amount of training of the skiers seems have influenced more on anaerobic threshold on maximum oxygen uptake. This result is in d agreement with our observations concerning training of Finnish cross-country skiers.

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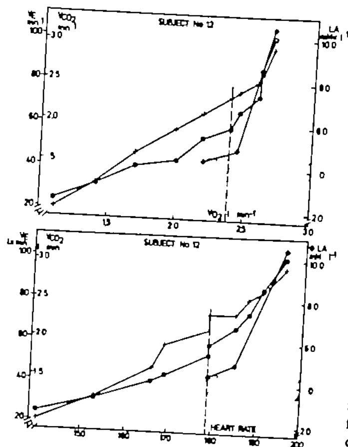


Fig 1 Example of anaerobic threshold (AT) determination To determine AT V V_{CO_2} and blood lactic acid values were plotted against corresponding V (upper curves) and heart rate (lower curves)-values Dashed vertical line denotes AT

the blood lactate concentrations of subject 1 were 0 8 and 1 5 mM after the 5th and 7th work loads Because the maximum blood lactate concentration of subject 1 was 5 0 mM (4 months later 5 6 mM) and that of subject 12 10 4 mM the overall capacity of the body to produce energy anaerobically could explain the observed differences in blood lactate levels during submaximum exercise Accordingly the abrupt increase in blood lactate concentration together with the respiratory responses might be more feasible than a rigid 4 mM threshold as a criterion of anaerobic threshold

It has been proposed that during prolonged submaximal exercise the most important determinant of performance might be the oxidative capacity of the muscles (e g Holloszy 1967 Costill et al 1976 Fink et al 1977 Rusko et al 1978) In this study the relationships between anaerobic threshold maximum oxygen uptake and selected variables of oxidative capacity of muscles were investigated The high activities of oxidative enzymes could increase the capacity of the muscles to ox... pyruvate during submaximum exercise when the oxygen supply to the working muscles is not probably adequate Consequently the reduction of pyruvate to lactate might be decreased and the increases in muscle and blood lactate concentrations occur at higher intensity of exercise or the anaerobic threshold is at higher level From the enzymes studied SDH and CS activities and the ΣOE correlated significantly with anaerobic threshold while max V_{O} showed no significant correlations with the enzyme activities.

The results seem to support the hypothesis that the submaximum or prolonged work capacity expressed as anaerobic threshold might be related to the oxidative capacity of the muscles During maximum exercise the supply of oxygen to the working muscles may be inadequate The insignificant correlations between max V_{O_2} and the oxidative enzymes studied seem to show that max V_{O_2} might more likely be related to central or local factors of circulation than to the oxidative capacity of the muscles (See Clausen 1977 Saltin 1977 Rusko et al 1978)

In previous studies significant correlations between max V_{O} and oxidative enzyme activities and muscle fiber composition have been observed (Costill et al 1976 Rusko et al 1978 Bergh et al 1978) These correlations might have been resulting from the heterogeneity of the subjects In our study the subjects were all endurance athletes and corresponding correlations were not observed The different deterioration rates in max V_{O_2} and the enzyme activities during detraining (Henriksson & Reitman 1976 Örlander et al 1977) may partly explain the lack of correlations between these variables in our group which was measured 4 months after intensive training and competition season Moreover the bicycle ergometer exercise and the muscle studied (m VL) are not necessarily the best means to characterize the effects of training in cross-country skiers

According to our hypothesis a high correlation should exist between anaerobic threshold and oxidative potential of the muscles Other factors in addition to oxidative capacity of the muscles might also determine the level of anaerobic threshold e g (1) rate of lactate transport from exercising muscles to the blood and the removal of lactate from the blood by heart liver and nonexercising muscles ... g capacity ...

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locomotion of the low spinal cat
oordination within a hindlimb

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FORSSBERG H GRILLNER, S & HALBERTSMA J Locomotion of the low spinal cat. I Coordination within hindlimb. Acta Physiol Scand 1980, 108. 269-281 Received 20 June 1979 ISSN 0001-6772. Department of Physiology III Karolinska Institutet, Stockholm and Department of Physiology University of Göteborg, Sweden.

Kittens were subjected to complete transection of the spinal cord (Th 10-12) 1-2 weeks after birth A few days after the operation they could perform alternating limb movements and somewhat later walking movements with thel hindlimbs on treadmill. The stepcycle of the hindlimbs could be divided into flexion phase (F) and a first (E_1) second (E_2) and third (E_3) extension phase The duration of the support phase decreased markedly with treadmill velocity while the swing phase decreased to much smaller xtent. The pattern of electromyographical activity in hip, knee ankle and toe muscles during treadmill locomotion was very similar to that of the intact cat. This related to both the timing and the general shape of locomotor bursts. The extensor muscles were thus activated well before the placement of the foot and able to produce enough force to support the body The propulsive thrust in each step was, however decreased and the animals showed more severe deficits particularly in their equilibrium control It is concluded however that neural networks in the spinal cord (with its peripheral inflow intact but without supraspinal influences) have the capacity to generate a specific and detailed locomotor pattern

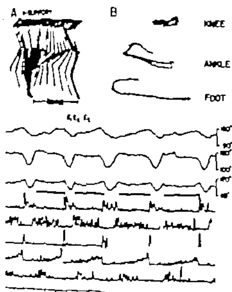
Key ords Spinal cord, spinal transection locomotion

neural control of locomotion can be divided 3 conceptually different tasks, (i) to generate stereotypic locomotor movements that result in propulsion typical for a given species (ii) to pt these movements to the environment and the ls of the animal and (iii) to maintain the equilib-m of the animal during the different body posi-ns encountered during locomotion. The aim of present study is to in estigate the extent to ich the cat spinal cord can reproduce reotypic locomotion (i). It is evident that the her types of anticipatory control implied under is excluded in the movement repertoire of the nal animal, and furthermore that the equilibrium ntrol must be drastically reduced

Eckhorst & Naunyn (1874), Freusberg (1874) and ltz & Freusberg (1874) described that dogs with inal cord transection in the lower thoracic region uld not only support themselves but also produce ythmical sequences of flexion and extension of e hindlimbs constituting reflex steps The epping could be induced by various stimuli but so occurred spontaneously if the animal was held with the limbs extended. In 1905 Phillipson analyzed films obtained from walking spinal dogs and defined the different flexion and extension phases, found in normal animals (see below) The similarity of spinal stepping to normal walking was, however questioned by many investigators (Magnus 1924 ten Cate 1940) Using different training or manipulati e procedures later investigators have been able to improve the walking movements in different kinds of chronic spinal animals (Kellog et al 1946, Shurrager & Dykman 1951 ten Cate 196., 1964 and Steltzner et al. 1975). The reports are however formulated as rather general descriptions with no effort to define how complete a walking pattern the spinal cord can generate Noradrenergic precursors or receptor stimulators may also release spinal stepping or walking on a treadmill in acute spinal animals (Grillner 1969 Budakova 1973 Forssberg & Grillner 1973). In this context it is relevant to note that an interneuronal network in the cat s spinal cord has been described which,

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Movements and muscular activity during slow ... In *A* the forward movement of the right ... presented as ... stickdiagram. The position of the ... presented as stick figures throughout the stepcycle ... intervals. In *B* the knee, ankle and foot trajec... are displayed at 6 ms intervals during 6 consecutive ... cles. The positions are related to the position of the ... which is fixed in the horizontal direction. In *C* the ... movements of the hip, knee and ankle (at the top) ... played together ... with the simultaneously recorded ... of TA, LG, St, Q and GM. The EMG' are filtered ... fied. The swing and support phases are also indi... in the stick diagram. The onset of flexion and the ... extension phases are indicated in the angular ... ments of the ankle (Phillipson 1905). The support ... are also indicated by schematic blocks. In the time ... at the bottom large lines represent seconds and small ...

...corder (Tandberg and Co, frequency response 1 250 ...

...fies. Sequences were chosen from the magnetic ... The position data ... were corrected for distortion ... errors and other nonlinearities of the Selspot sy... ...ing data could be interpolated. The position data ... then smoothened by software filters, which cut off ... ...ers abov 5 H for the pelvis and hip and above 10 ... the ankle and the tarsometatarsal joint. The pivot ... of the knee was calculated by triangulation from the ... the ankle. The movements of the limb could then ... played on ... graphic terminal (Tektronix 4010) as ... diagrams (see Fig 1A) with straight lines between ... ...ents representing the position of each LED or as ... ...tories (see Fig 1B) of the LEDs on the foot ankle ... in relation to the LED on the pelvis, which then ... "fixed" in the horizontal direction. The angles of the ... knee and ankle joints were calculated by ... cosine formula, and the flexion–extension movements of the joints could continuously be displayed on the terminal (see Fig 1C).

The data from the ADC (EMGs and forces) were usually also smoothened by a software filter (<30 Hz) before they were displayed together with the stick diagrams or with the movements of the joints (see Fig. 1C). A cursor was manually used to mark different events in the step cycle as the onset and termination of the EMG-bursts or the onset of the flexion and extension phases in the angular movements of the joints. In this way the duration of different phases as well as the relation of differnt events in different muscles and joints could be calculated and treated statistically. I order to estimat the amount of muscular activity the rectified EMG bursts were integrated and the mean and standard deviation (S.D.) of the total activity during several step cycles were calculated. The mean and the peak intensity of each locomotor burst were also calculated. As there was some variation in the appearance of the locomotor bursts also at regular walks the bursts could be averaged. The step cycles were determined by the movements of one joint (ankl) where the onset of any phase previously marked by the cursor from the angular movements (see above) could start and terminate the cycle. The step cycles were first normalized, whereafter the mean and S.D. of the electrical activity in each instance of the cycle could be calculated and displayed on the graphic screen (Fig. 3). Any point of movement or EMG-traces could be used as trigger points for the averaging.

Intact cats. The obtained data have as far as possible been compared to published reports on normal locomotion. Howeve in some instances no comparative data have been available. Three intact cats were therefore trained to walk on the same treadmill and were subjected to the same procedure as the spinal animals (see above).

Abbreviations. The following abbreviations are used: Flexion phase F first second and third extension phase E, E_2 and E_3 respectively m extensor digitorum brevi EDB m. gastrocnemius lateralis, LG m. flexor digitorum or hallucis longus, FDL, m. soleus, Sol, m. tibialis anterior TA, m. quadriceps (vastus lateralis or intermedius) Q m. semitendinosus, St; m. gluteus medius GM m. adductor magnus, AM, m. iliopsoas, IP m. sartorius (medial part), Sart; light emitting diode LED; electromyography EMG standard deviation, S.D. analogue digital converter ADC.

RESULTS

The spinal kittens exhibited some muscle tone in their hindlimbs 1– days after the transection of the cord and showed alternating flexion and extension movements when lifted in the air with the trunk held vertically. Left alone on the floor the young kittens dragged themselves forward with paddling movements of the forelimbs. The hindlimbs were then held extended or flexed or could perform alternating movements. The latter type of movement would occur particularly if the cats rotated the

without afferent feedback can generate a complex pattern of flexor and extensor activity (T G Brown 1911 1914 Jankowska et al 1967*a b* Grillner & Zangger 1978 Vidal et al 1979) Peripheral feed back acting on this network has also been reported (Grillner & Rossignol 1978 Andersson et al 1978)

In comparing the walking movements of spinal and decerebrate cats Sherrington (1910) found large differences especially during the "extensor phase" of the step He believed that a mechanism essential to produce "real walking was situated "between the levels of anterior colliculus and hind er edge of pons"

In this study the locomotion of chronic spinal kittens walking on a treadmill is analyzed i.a by recordings of the movements and of the elec tromyographical activity in the different hindlimb muscles The specific pattern of the spinal locomo- tion is then compared to data from walking in the intact cat (e g Engberg 1964 Engberg & Lundberg 1969 Goslow et al 1973 Rasmussen et al 1978) or recordings obtained from intact cats walking on the same treadmill In a first study the characteristics and the timing of the angular movements and mus cular activity of one hindlimb are investigated In a second the coordination between the two hindlimbs is reported Some of the results have been mentioned previously (Forssberg Grillner & Sjöström in Grillner 1973)

METHODS

General procedures Fourteen kittens from seven litters were subjected to a transection of the spinal cord at a low thoracic level from the 6th to the 17th day after birth The spinal cord was exposed by a laminectomy the dura was opened and half a segment of the cord was removed whereafter the dura was sutured again The spinal cord was dissected out after the animal was sacrificed and the level and the completeness of the lesions was checked In all kittens there was a complete lesion between Th 10– Th 1

The kittens were brought back to their mothers some hours after the operation and were then nursed and fed by her The increase of the body weight was followed each day as an indicator of the general health of the kittens While the mothers usually succeeded in emptying the bladder of the kittens by licking around the perineum the bladder of each kitten was checked twice daily and man ually emptied when needed Infections usually from the urinary tracts were treated by bensylpenicillin or ampicil lin The kittens were "trained" daily 10 to 20 min on a treadmill

Recording procedures EMG (electromyography)-elec trodes and LEDs (light emitting diodes) for [illegible] recording (see below) were fixed to the kitten in [illegible] brief Halothane anesthesia. During the experiments [illegible] fore part of the kittens were either carried by one [illegible] the investigator or were standing with the forelegs [illegible] plat form The hindlimbs were on a treadmill belt [illegible] was motordriven and could be moved at various [illegible] speeds The tail was gently held with the other hand [illegible] assist the kitten to maintain equilibrium

EMG recordings Pairs of thin copper [illegible] insulated except for 1 mm at the tip were inserted [illegible] desired muscles with hypodermic needles (Engberg & Lundberg 1969 Forssberg & Grillner 1973) The [illegible] position was checked by stimulation of each [illegible] against the ground electrode which [illegible] placed at the back and simultaneous palpation of [illegible] contracting muscle The signals were pre-amplified [illegible] P 15) and high pass filtered (5th order [illegible] eliminate movement artefacts They could be [illegible] recorded on an inkwriter and on a tape recorder [illegible] rectified and low pass filtered (5th order Bessel [illegible] Hz) and fed to a minicomputer (see below)

Movement recordings The movements [illegible] by a Selspot system (Selective Spot Recognition [illegible] Selcom AB Partille Sweden), with a sampling rate [illegible] but for our purpose reduced to 156 Hz. Five [illegible] LEDs (light emitting diodes) were glued to the [illegible] limb at the pelvis (iliac crest) hip, knee ankle and [illegible] tarsometatarsal joint. The light pulse from each diode [illegible] detected by a special optoelectronic detector [illegible] the focal plane of a camera and the position [illegible] with processing electronics The x and y coordinates [illegible] available in digital form to be fed into the computer [illegible] for some LEDs in an analogue form. Attention [illegible] to avoid reflexions in the floor and walls from the [illegible] and other infra red radiations

Recordings of force reactions. The reaction force [illegible] ground to the foot during the support phase was [illegible] on a piezoelectric force plate (Kistler model [illegible] The kitten was held in a similar way as when [illegible] the treadmill but stepped instead on the force plate [illegible] vertical force (Fz) and the horizontal forces in the [illegible] gitudinal (Fy) and transverse (Fx) direction [illegible] corded on the inkwriter and the tape-recorder and [illegible] into the computer Before entrance to the computer [illegible] signals were low pass filtered (5th order Bessel [illegible] Hz)

Storage of data The low pass filtered signals [illegible] muscular activity and of the force reactions were [illegible] to digitally converted with a sampling rate of [illegible] Special hardware was developed to control the [illegible] and synchronize it to the Selspot system All the [illegible] fed to a minicompute (HP 21MX) and temporarily [illegible] on disc In this way the data could be [illegible] obtained during 90 s Directly after each recording [illegible] (normally 10 to 30 s) the data were stored on a [illegible] tape and new recordings could start

The raw (unfiltered and not rectified) EMG [illegible] analogue signal from the force plate and usually [illegible] analogue signals of the x and/or y coordinate [illegible] LED glued to the toe could be recorded on an [illegible] (Mingograph frequency respon [illegible] 50 Hz) and [illegible]

...to extend during the end of E_1. After foot ... the hip continued to extend while the knee ... ankle yielded (flexed) under the load (E_2) to ...ently extend again (E_3) (see Fig. 1C and ... Legend). During slow walk the extension in ... and ankle at the end of the support phase ... weak (see Fig. 1C and Fig. 4A) which ... difficult to determine the transition from E_2 ... and, therefore, these two phases have been ... together in the plots. Knee and ankle thus ... closely linked throughout the stepcycle ... the hip proceeds in monotonic flexion–ex... movements in phase with knee and ankle ... E_1–F but out of phase during E_1–E_3.

...adjustment in the stepcycle

...elocity of the backward movement of the limb ... the support phase was controlled by the ... of the belt. During faster walks the duration ... stepcycle shortened, from 880 ms at 0.14 m/s ... the half (450 ms) at 0.70 m/s (see Fig. A). ...reduction was mainly due to a shortening of ...pport phase from 570 ms to 190 ms, whereas ...rward swing was reduced to a much smaller ... (with 60 ms, see Fig. A). When the swing ... was subdivided into F and E, F was found to ... only slightly (160→190 ms) but E some... more (150→100 ms). In relative terms the ...tion of the stepcycle taken up by E_2+E_3 (i.e. ...pport phase) will decrease with speed and F ... (Fig. 2B).

...amplitude of the angular movements of the ... especially those of the knee and ankle, in... at faster speeds (Fig. 2C). The amplitude of ...gitudinal movement of the foot during the ... phase increased about 66% from 0.07 m/s ... m/s in one kitten. The lengthening was main... to the increased joint movements of the limb ... to a much less degree to movements of the ... The ankle had a tendency to hyperextend in ... kittens (see Fig. C). This was probably due ... passive torque at the end of the support (see ...ssion) and a weakness of the flexor muscles.

...activity in the extensor muscles shortened at ... speeds, in parallel with the support phase ... 700 ms to 300 ms, see Fig. D). In contrast, ... duration of the knee flexor activity increased ... but at the lowermost speeds to remain ap...ximately constant (60–140 ms, see Fig. D cf ... phase in Fig. A). The total amount of muscle ... activity was approximated by integration of the rectified and filtered EMGs (see Methods). In the knee flexor St it increased steadily as the kitten walked faster (see Fig. 2E). At lower speeds this was mainly due to the increase of the duration (see Fig. 2D) but at higher speeds to the increased intensity (see Fig. 2F). In the extensor muscle Q the activity decreased to 40% at faster speeds (Fig. 2E). This was not only due to the marked reduction

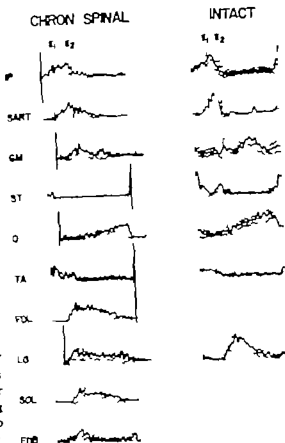


Fig. 3. Averaged EMG from spinal and intact cats. The rectified and filtered EMG from the muscles indicated are averaged after normalization of the stepcycle duration. The mean is indicated as continuous line and the S.D. as dotted lines. The recordings are from different cats (chron. spin. = 2; intact = 2) and from different experimental sessions but may still be comparable since the cycle durations are chosen to be around 800 ms (±20 ms) and the onset of the stepcycle is triggered by the movement in the ankle where the onset of the flexion and the first extension phases have been used as trigger points. The different phases are marked as lines. The number of averaged locomotor bursts vary between 8 and 12. The position of the FDL electrode (see abbrev.) was confirmed by the forceful digit flexions elicited by each stimulus (see Methods).

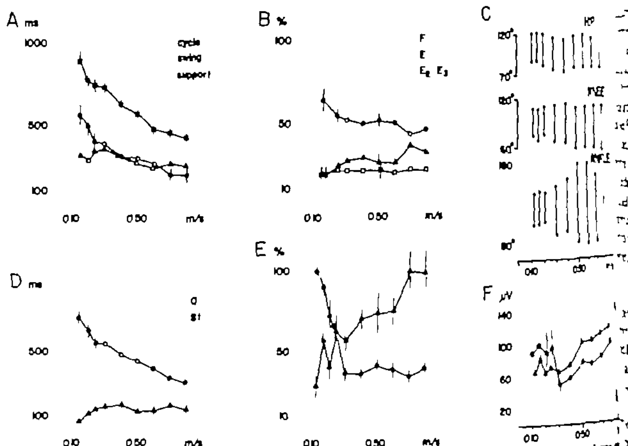


Fig 2 Adjustments in the stepcycle with speed The data points are collected from one spinal kitten during a experimental session during different speeds of walk but are representative for the majority of the kittens. In A the m and S D (exceeding 20 ms) of the durations of the stepcycle of the swing phase and of the support phase are plotted Symbols indicated in A, B and D (D is also applicable to E and F) In *B* the mean and S D (exceeding 5%) of the first phase the first extension phase and of the sum of the second and third extension phases are represented as percentage of total stepcycle duration at different speed In *C* the extremes of the angular movements are shown for each speed shows the durations of the locomotor bursts of the extensor (Q) and the flexor (St) of the knee (mean ± S D (exceeding 20 ms)) and *E* the total amount of the electrical activity during the locomotor bursts calculated by integration of the rectified and filtered EMGs (mean ± S D) In order to compare the two muscles in the same plot the maximal value mV s for each muscle is defined to 100% In *F* the means and S D s for the mean intensity of the locomotor bursts plotted The numbers of observation vary from 8 to 14 in each data point

trunk, so that both hindlimbs pointed towards one side As the kittens grew older they would support themselves with the forelimbs held vertically as ordinary cats do The hindlimbs were kept as above or could occasionally be used to support the animal for several consecutive steps before it fell to one side or the other If however the hindquarters were prevented from falling by gently holding the tail the kittens could support their body weight and the hindlimbs participated in walking. Placed on a treadmill the kittens could adjust to the belt speed (cf Forssberg & Grillner 1973 Budakova 1973) Young kittens could track the treadmill over a rather wide range of velocities but it has been our impression that the older preparations would produce "good walking" only in a more narrow speed range All kittens could walk i.e perform alternating hindlimb movements (as in walk or trot), and some of them would switch over to a more simultaneous activation of the limbs as in gallop at higher treadmill speeds. The coordination between the two hindlimbs will be dealt with separately (Forssberg et al 1980)

The structure of the step cycle During treadmill locomotion (see Methods) the foot was placed on the belt and the limb then moved backwards in relation to the body during the support phase

The swing phase started with a simultaneous flexion phase (F) in the hip knee and ankle and continued with an extension of the knee and ankle (the 1st extension phase (E_1)) while the hip continued to flex (see Fig 1 ig 4 A and B)

...to extend during the end of E_1. After foot ... the hip continued to extend while the knee ... ankle yielded (flexed) under the load (E_2) to ...ently extend again (E_3) (see Fig. 1C and Legend). During slow walk the extension in ...e and ankle at the end of the support phase ...en ...eak (see Fig. 1C and Fig. 4A) which ... difficult to determine the transition from E_2 ...and, therefore, these two phases have been ... together in the plots. Knee and ankle thus ... closely linked throughout the stepcycle ...s the hip proceeds in monotonic flexion–ex... movements in phase with knee and ankle E_3-F but out of phase during E_1–E_2.

...adjustment in the st...pcycle

...locity of the backward movement of the limb ... the support phase was controlled by the ... of the belt. During faster walks the duration ... stepcycle shortened, from 880 ms at 0.14 m/s ... the half (450 ms) at 0.70 m/s (see Fig. A) ...eduction was mainly due to a shortening of ...pport phase from 570 ms to 190 ms whereas ...rward swing was reduced to much smaller ... (with 60 ms, see Fig. A). When the swing ...was subdivided into F and E_1, F was found to ...e only slightly (160→150 ms) but E_1 some... more (150→100 ms). In relative terms the ...tion of the stepcycle taken up by E_2+E_3 (i.e. ...pport phase) will decrease with speed and F ...se (Fig. 2B).

... amplitude of the angular movements of the ... especially those of the knee and ankle in...ed at faster speeds (Fig. 2C). The amplitude of ...agittal movement of the foot during the ...rt phase increased about 66% from 0.07 m/s ... m/s in one kitten. The lengthening was main... to the increased joint movements of the limb ... a much les... degree to movements of the ... The ankle had a tendency to hyperextend at ... (see Fig. C). This was probably due ... passive torque at the end of the support (see ...nsion) and a weaknes... of the flexor muscles.

...e activity in the extensor muscles shortened at ...er speeds, in parallel with the support phase ... 700 ms to 300 ms, see Fig. 2D). In contrast, ... duration of the knee flexor activity increased ...ewhat at the lowermost speeds to remain ap...ximately constant (60–140 ms, see Fig. D cf ...ephase in Fig. A). The total amount of muscu... activity as approximated by integration of the

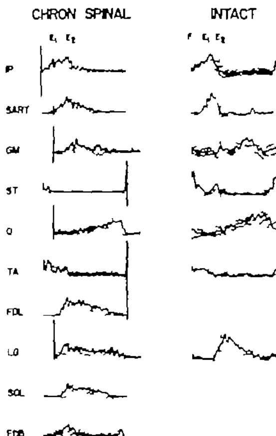


Fig. 3. Averaged EMG from spinal and intact cats. The rectified and filtered EMG from the muscles indicated are averaged after normalization of the stepcycle duration. The mean is indicated as continuous line and the S.D.s as dotted lines. The recordings are from different cats (chron. spin = intact =2) and from different experimental sessions but may still be comparable since the cycle durations are chosen to be around 800 ms (±20 ms) and the onset of the stepcycle is triggered by the movement in the ankle where the onset of the flexion and the first extension phases have been used as trigger points. The different phases are marked as lines. The number of averaged locomotor bursts vary between 8 and 12. The position of the FDL electrode (see abbrev.) was confirmed by the forceful digit flexions elicited by each stimulus (see Methods).

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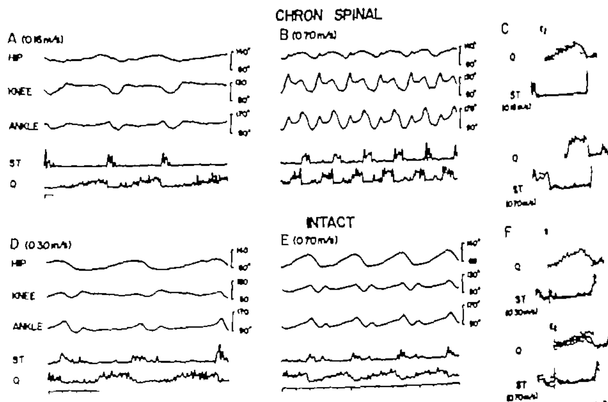


Fig 4 Comparisons of the changes in locomotor activity at different speeds in chronic spinal and intact cats A B
E show the angular movements of the hip knee and ankle and the EMG s from the flexor (St and the extensor (Q
knee during slow (A D) and fast walk (B E) from a chronic spinal and an intact cat respectively In *C* and *F* the
and filtered EMG's are averaged from the actual sessions (n=8–12) Q is triggered by the beginning of E_1 and St
onset of F Note that the early Q burst is fully represented (i.e ± S D) only after the main Q burst (C and F) N
that the extension of the hip of this spinal cat is braked at foot placement at higher speed (B) (see Discussion) I
velocity of the belt is indicated for each recording

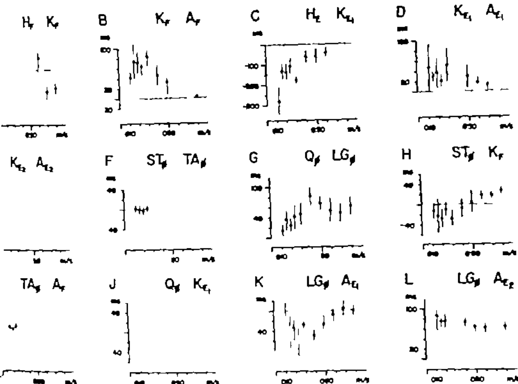
of duration with speed (Fig 2 D) but also to a fall of the mean activity in the middle range (Fig 2 F) The mean intensity level at different velocities showed in fact two different slopes At low speeds it was kept at a constant high level but at 0 30 m/s it suddenly fell to slowly increase again at higher speeds (Fig 2 F) This change occurred at the same approximate speed as the changes in the pattern of the Q-burst (see below and Fig. 4 A–C) but a similar tendency was also seen in the ankle extensor

Muscular activity during the stepcycle

Fig 3 shows the EMG activity for a number of muscles in the chronic spinal cat and for comparison recordings from intact cats walking on the same treadmill under the same conditions In the Result section we will however only deal with the reults from the spinal preparations

Extensor muscles The ankle extensors had an intense onset during E_1 with a maximal activation around the shift E_1–E_2 The intensity of the burst then continuously decreased to terminate in the end of E_3 (see Fig. 3) The general shape of the EM curve was invariant with speed though the ov intensity increased No distinct differences w found between the different ankle extensors (L Sol) and the foot extensor (FDL) regarding t timing or the shape of the bursts In the recordi from some kittens still walking well a burst c curred in LG only at the transition of E_1–E_2 l corresponding to the usual peak of the EMG b

The pattern was different in the knee extensor At lower speeds Q started smoothly during F continuously increase until the end of E_3 whe abruptly terminated (Fig 3 and 4 A and C). At h speeds the pattern changed entirely A small b appeared in the beginning of E_1 or the end of F Th burst was usually isolated from the more sp wave formed and much larger burst during F a E (Fig 4 B and C) The separation of the Q-acti in two bursts could be found in all kittens and w the only pattern seen in some even at slow sp I a few kittens the second burst was very short a occurred at the end of



Correlation of different movement and electromyographical data at different speeds. The onset of the flexion and [illegible] phases in different joints and the onset of the locomotor bursts in different muscles are correlated to each [illegible] The mean and S D of the differences are plotted in ms at different speeds. All data are from one spinal kitten, [illegible] one experimental session (the same as in Fig. 2) and the observations for each data point vary between 8 and 14 [illegible] Symbols used are H hip, K knee A ankle and the index F E_1 and E_4 the onset of the flexion, the first and the [illegible] extension phases respectively St, TA Q and LG see abbreviations Ø indicates the onset of the locomotor [illegible] the muscles A thus show [illegible] hen the hip flexion occurs in relation to the knee flexion. A positive value means [illegible] flexion precedes knee flexion. A–E show the onset of different phases of movements at different joints in relation [illegible] other and F and G the onset of the flexors and [illegible]xtensors of the knee and ankle in relation to each other At higher [illegible] the onset of Q is counted from the first Q burst H–L show the relation between muscle activity and movement.

[illegible] hip extensors studied gluteus medius and [illegible] ctor magnus had [illegible] smooth onset during E_1 [illegible] activity was usually maximal during E_4 but the [illegible] of the activity was more variable than in the [illegible] extensor muscles (see Fig. 3).

[illegible] lexor muscles The flexors of the ankle (TA) [illegible] the knee (St) were usually synchronously acti[illegible] during the [illegible] ery end of E_3 (Fig. 3 and 5 F) St [illegible] very sharp onset and lasted only during the [illegible] part of F [illegible] hile TA had [illegible] somewhat smoother [illegible] et and was active throughout F A second burst [illegible] activity was found in St during E at slower walks [illegible] about 50 [illegible] of the cat (see also Forssberg et al [illegible], Fig 1 A). The size of the second burst in[illegible] sed with [illegible] peed and at least a small peak of [illegible] ivity could be seen in all spinal preparations at the fastest speeds (Fig. 4 B). A second burst or peak of activity during E was never found in TA

The hip flexor IP started more gradually than the distal flexor muscles to reach a maximum later in the stepcycle and cease approximately at the end of E Sartorius started later but reached its peak at the same time as IP In some kittens an additional small burst more or less isolated from the main burst could occur at the end of E_3

The short foot dorsiflexor (EDB) displayed a large burst in E_1 and some residual activity throughout E_2 [illegible] nd E_3 (Fig. 3)

Coordination [illegible] antagonistic muscles An appar ent finding was the strict reciprocal activation of the extensor and the flexor muscles of the knee The sharp termination of Q at the end of E was

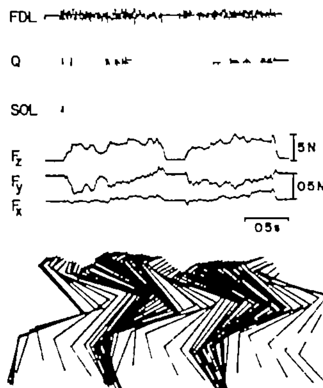


Fig 6 Reaction forces produced during overground locomotion The spinal kitten is held in a way that the right hindlimb is stepping on the forceplate The movements are displayed as a stick diagram (see Fig 1 A) where the pelvis is displayed at a constant speed forward (to the right) synchronized to the recordings of the reaction forces and of the EMG's F is the vertical force The horizontal forces are divided into longitudinal components (F) where the forwardly directed reaction force is displayed upwards and in transversal components (F) where left is upwards. Note time and force calibrations

always followed by as sharp an onset of St with a delay of 5–20 ms independently of the speed St was never activated before the activity in Q had ceased (unfiltered EMG s) The shift during E from St to Q-activity was not as constant At slow walks there was a longer delay (120 ms at 0 12 m/s) than in faster (15 ms at 0 80 m/s) The second smaller burst of St which occurred more regularly at higher speeds had a tendency to occur during the pause which separated the first Q-burst from its main burst

There was not as distinct a reciprocity between the antagonists of the other joints TA started after the smooth termination of LG During E however LG could be activated before TA had ceased (Fig 1C) Also at the hip joint there was an overlap of flexor and extensor activity

Relations between the muscular activity and the movement at different joints and at different speeds

During slow walk the onset of flexion of the hip and knee started approximately simultaneously speeds During slow walk the onset of flexion of the hip and knee started approximately simultaneously although there was a large variability (Fig 1C ... In the ankle joint the shift from extension to flexion was more distinct (Fig. 1C 4A) but it occurred 40–80 ms after the flexion of the knee at slow ... (Fig 5B) The flexor muscles of the knee and the ankle were however activated almost simultaneously (see above and Fig. 5F). The ankle flexion ... 20–40 ms after the onset of the activity in the flexor muscle (Fig 5I) i e the latency needed for effective electromechanical delay (Forssberg ... The knee however started to flex prior to ... activity in Q had ceased and that in St started ... 4 A and 5H) This means that the ankle joint ... lowed the centrally generated efferent ... while the knee joint went into flexion despite ... ing extensor activity The knee extensor torque ... thus not sufficient to counteract the external ... (see Discussion) At faster walks the knee flexor could however contribute to the onset of flexion ... the knee (Fig. 5H) as St-activity then started ... the flexion There was a more distinct ... during E_3 and a sharper transition into flexion ... 4B) The delay between knee and ankle flexion ... practically abolished at the highest speed (Fig ...

The knee and the ankle joint went into extension again in the middle of the swingphase and also ... this shift the knee was leading (Fig 5D) The ... extensor was as a rule not activated before the ... (Fig 5K) and the knee extensor only at the higher speeds (i e the first Q burst Fig. 5J), which indicates that the extension in both knee and ankle ... least at lower speeds may be passively induced ... the inertia of the moving limb The hip kept flexed during the largest part of E and its extension occurred after the knee (Fig 5C) and after the ... tion of activity in the extensor muscles ... Fig 1C)

When the foot was placed on the belt both knee and ankle was seemingly prevented from any further extension and as one would expect E_3 started simultaneously in the two joints (Fig 5E). The ankle extensors were always active at least ... prior to the placement of the foot at all speeds (Fig 5L) i e well in time to ... tension to meet

...al load of the body (see Discussion). When Q ... moothly activated at slow walks the onset of ... tion always preceded E_1 (Fig. 4C). When ... ited with a first isolated small burst, in E or ... times even in F the larger burst was usually ... ng at around the placement of the foot, but ... In some cats regularly be activated 5–15 ms ... foot contact (Fig. 4C).

...proved locomotion. It may be asked if the ... imbs in addition to carry the weight of the ... uarters can produce any propulsive force ... an unspecific stimulation of the skin such as ... zing the tail somewhat it was possible to en... the locomotor activity (cf. Sherrington 1910) ... g. 6 the front part of the body was carried by ... and, and slightly moved forward in a way that ... ated the right hindlimb to be placed on a force ... during the support phase. The extensors pro... d force enough for one limb to carry most of ... eight of the hindpart (55% of the total body ... ht). In spite of the large vertical force the body ... t the end of the swing phase and at the begin... of the support phase. This was due to an ... metry of the two limbs where the contralateral ... was weaker than the recorded one and could ... produce sufficient force. During the beginning ... he support phase the limb was decelerated as ... n by the backwardly directed reaction force ... The forwardly directed reaction force which ... ng normal locomotion propels the animal for... d, ... as, however, very small and could only ... duced after stimulation of the tail. It is also ... worthy that the spinal kittens could drive the ... dmill belt at higher speed when the friction ... ces of the belt were balanced.

...CUSSION

...t an intact animal increases its speed of propul... it might do so by progressively enhancing the ... el of activity in the neurons responsible for ... erating the locomotor activity. Thus, in ... ncephalic preparations locomotion may be in... ed from particular regions in the brainstem. Low ... ngth stimulation (e.g. at 50 Hz) may result in ... motion at slow walking speed. If the limbs are ... a treadmill, the speed of locomotion may be ... ified ... thus a considerable range by changing ... belt speed. If the strength of the brainstem ... ulation is increased the cat may trot and if further ... creased, gallop. The extra force needed is gener...

ated by recruitment of new motorunits (Shik et al 1966, Severin et al. 1967). The situation in the spinal cats is fundametally different as the excitability of the cord cannot be modified by descending signals. In these animals the relevant spinal circuits have a constant excitability that clearly is high enough to elicit locomotion when the treadmill is driven within a rather large range of treadmill speeds. It is possible that the extra unspecific afferent input caused by the movements may give some extra drive to the generator network (see Grillner & Zangger 1979). It is reasonable to regard the spinal network as set for a very low speed which is also in accordance with the finding that the hip extensor torque is small (Fig. 6). The fact that the movements adapt to the speed of the treadmill must rely on feedback from the moving limbs. There are several peripheral inputs that can serve to regulate the duration of different phases of the step (Pearson & Duysens 1976, Grillner & Rossignol 1978, Andersson et al. 1978).

The striking dependence on speed of the duration of the support phase (see Fig. 2) must thus be accomplished by the reflex input presumably in a similar way to that occurring in the intact animal (Arshavsky et al. 1965, Goslow et al. 1973). The yield of the knee and the ankle that occurs in the support phase is to a large extent due to the load on the limb that in the intact cat becomes maximal at the peak of the yield (Phillipson 1905, Grillner 1972, cf. Walmsley et al. 1978). The torque value around a joint at any instance depends i.a. on the load on the limb and the distance between the joint axis and the resultant of the force vectors (inertia neglected). The muscles should produce an equal torque opposing the external forces. It is clear that any effects such as a slight deviation of the resultant vector may lead to a marked change in the torque required. One minor abnormality of the spinal kittens (at low speeds) is the fact that the knee flexion at the end of the support starts too early i.e. before the knee flexors have started to become active. This may be due either to that the required torque is higher than normal (see above) or that the degree of extensor activity is to low.

It is interesting that the duration of the flexion phase decreases slightly with speed. To accomplish this the flexion torque must increase with speed particularly as progressively more torque will be required to reverse the direction of the entire limb. This results from a marked increase in the degree of flexor activation (Fig. E). What induced this in

crease in flexor activity? It would seem probable that it is related to events in the ipsilateral rather than the contralateral limb If so it must be a process that occurs prior to the end of the St-burst and presumably before it has started It is conceivable that events in the preceding phase could be the determining factor such as the duration of the support phase or the interval of the extensor activity or the velocity of extension

There was also a substantial lengthening of the amplitude of the leg movement with speed in the spinal kittens Such a lengthening may however also occur in intact cats Calculations of the support length (i e the distance the animal moves during each support phase) from the graphs of Goslow et al (1973) reveal a lengthening of 18% from slow walk (0.5 m/s) to fast walk (0 9 m/s) and even 35% to fast trot (? 7 m/s) but their later study (Wetzel et al 1976) comparing only rather fast walks (1 m/s) and trots showed very small differences Lengthening occurred also at low speeds in our intact cats walking on the same treadmill as the spinal kittens (40% from 0 1 m/s to 1 m/s) and in intact dogs (Arshavsky et al 1965 28% from 0 9 m/s to 3 m/s)

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e locomotion of the low spinal cat
nterlimb coordination

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The interaction of the two hindlimbs were investigated by an analysis of the muscular activity and the movement in 14 chronic spinal kittens during treadmill locomotion (in kittens subjected to transection of the spinal cord (Th) one or two week after birth) At low speed the limbs were alternating (alk or trot) At higher they were activated more simultaneous as during gallop The tw limbs could alk at different velocities as during alking in circle hen the two belt of the treadmill were driv at different speed The duration of the support phases w mainly influenced by the speed of the belt on hich the limb as alking The limbs could still maintai common rhythm up to a tw or three fold speed difference as the flexion or the first extension phase of the limb alking on the "fast" belt as prolonged and the flexion phase of the slow limb was shortened. At extreme speed differences the limb on the "fast belt performed ... 3 and even 4 steps during one stepcycle of the slow limb The placement of the feet was found to maintain the most stable relationship during alternating gait at different speed differences It is concluded that all phases of the step cycle are modifiable and that there are several mechanisms coordinating the limbs ithin the spinal cord

Key ord Locomotion, spinal cord spinal transection interlimb coordination

Holst (1934 1935 1939) suggested on the basis series of elegant studies that the coord nation ween the different fins during fish locomotion lted from an interaction between independent ional centers controlling each fin. Von Holst 39) also pointed to the possibility that thi prin- le could apply to the coordination of the limbs in mals. This idea was developed further n a oretical model of quadrupedal locomotion (Gel- d et al. 1966), which in turn was supported by erimental evidence (Shik & Orlovsky 1965 ugin & Shik 1970 Grillner & Zangger 1979) erneurones, which may be responsible for the ordination between different generators ha e en identified in two invertebrate motor systems, e controlling walking in the cockroach and the her the swimmeret system of the crayfish (Pear n & Iles 1973 Stein 1971 1974 1976). These urones are active during the segmental motor sts and provide an efference copy which is mehow used to achieve intersegmental coordina- on In the swimmeret system studies on the effect f an indirect activation of the coordinating neurones have allowed the identification of certain properties of the coordinating system (Stein 1974 1976) Also in mammals (cat) part of the scending neuronal activity to the cerebellum provides effer ence copy ignals (Arshavsky et al 1972, 1978) Such signals could also be utilized t coordinate different autonomous network of the limbs (Grillner 1975) Until now there is however no mammalian work that so far has revealed the specific neuronal mechanisms of the interlimb coupling. E en on behavioural level little is known on the mode of interaction between these net works in different situations and in different animal preparations.

The spinal cord of the cat has the capacity to produce the detailed coordination of different muscle groups of limb which results in walking

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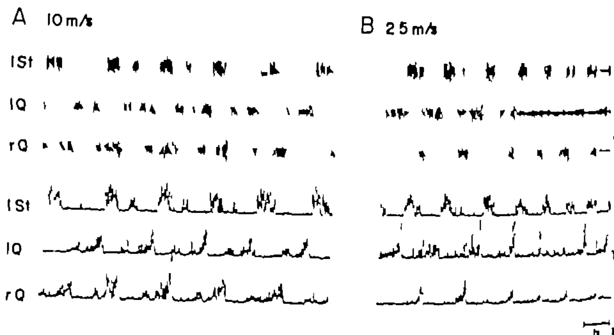


Fig 1 Muscular activity of a chronic spinal cat during treadmill locomotion The raw (amplified and high pass filtered) EMGs from the flexor (lSt) and the extensor (lQ) of the left knee and from the right knee extensor (rQ) are shown at the top and the rectified and filtered versions are shown at the bottom *A* Alternating locomotion at a speed of 1 m/s *B* Change over from an alternating to a nonalternating pattern at a speed of 2.5 m/s (From Tiger)

movements (Forssberg et al 1980) To induce such movements the supraspinal centers may only have to regulate the excitability level of the relevant spinal network But in addition each limb must be coordinated to all the other limbs In normal locomotion along a straight line despite large variations two main forms of hindlimb coordination can be observed alternate mode as in walking or trotting or a more or less simultaneous mode as in the different forms of gallop (Stuart et al 1973 Halbertsma et al 1976 English 1979) Another complex form of interlimb coordination occurs when an animal walks along a circular path and the outer limbs have to cover a longer distance (i e walk faster") than the inner ones The mesencephalic walking cats deal with such a situation by appropriate adaptations of the stepcycles on the two sides (Kulagin & Shik 1970) This was studied by having the cats walk with the right and the left pair of limbs on two treadmill belts the speed of which could be controlled independently

The present work describes how the lumbosacral spinal cord alone in chronic spinal cats manages to coordinate both hindlimbs when walking at different speeds under the situations discussed above Preliminary reports have been published (Forssberg Grillner and Sjöström in Grillner 1973 and Forssberg et al 1976)

METHODS

Four low (Th -Th) chronic spinal cats were used in the main part of this study but additional observations were also made on 10 more similar spinal cats The operation the training procedures and the recordings and data processing techniques were described fully in Forssberg et al (1980) In four of the cats the movements and electromyograms (EMGs) of the two hindlimbs were recorded during locomotion on a treadmill with "split belts" Both belts could be driven at the same speed or one could be disconnected from the motor and manually operated while the other was still driven by the motor The two limbs could thus be made to walk at different speeds The velocity of the belts was not always constant especially at lower speed and was therefore also estimated from movements of the diodes on the tarsometatarsal joint during the support phases The limb walking on the faster belt is called the "fast limb" and that on the slow belt the "slow limb"

Abbreviations The following abbreviations are used Flexion phase F first second and third extension phase E_1 E_2 and E_3 respectively m tibialis anterior TA m quadriceps (vastus lateralis) Q m semitendinosus St ST m iliopsoas IP m sartorius Sart standard deviation S D electromyogram EMG

RESULTS

1 *Treadmill locomotion*

Chronic spinal cats could walk on a treadmill at a speed adjusted to that of the treadmill belt and with

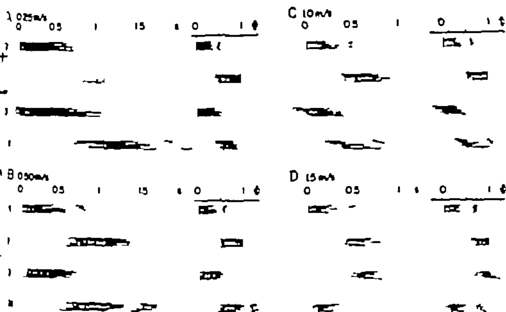


Fig. ? Schematical representation of the muscular activity from chronic spinal cat during treadmill locomotion at ... speeds. The duration of the EMG-burst of left lSt, and lQ and right rSt and rQ are measured from ... recordings on a graphic tablet (digitizer) connected to a desk calculator (HP 9830 A) and by plotter drawn ... in real time to the left (note time scale at the top) and normalized to the cycle duration to the right. The bursts of ... muscles from several consecutive stepcycles are plotted beneath each other. A cycle starts with the onset of ... activity of lSt. The end is marked with single dot. *A, B* and *C* show alternating gaits at increased speeds. *D* shows ... pattern as during gallop. (From Tiger.)

...locomotor pattern of each limb similar to that of ... cats (Forssberg et al. 1980). During slow ... the two limbs alternated, i.e. an event in one ... was delayed out of phase by half a stepcycle ... with the same event in the other limb (Figs ... A, B and C). In Fig. 6 (left column) the ... and the midpoint of the support phase (A) and ... the extension (B) and flexion (C) of the hip of ... hindlimbs are out of phase by 50 %. The onset ... the termination of the extensor activity were, ... not as symmetric (Fig. 6 D, left column) ... asymmetry was due to a difference in the burst ... (20 % shorter in the left limb). However ... midpoints of the period of support of the two ... were alternating (close to 50 %, cf. also 6 A ... B). The asymmetries between the limbs were ... in all spinal cats but at different degrees ... they could be more or less pronounced than ... is shown in Fig. 6. Behaviourly such asym... were manifested as limping (cf. Forssberg ... 1980). All cats ($n = 14$) displayed alternation ... wide range of velocities (cf. Fig. 2 A–C) ... the speed increased they ($n = 11$) could suddenly switch to another stable mode of interlimb coordination in which the two limbs performed simultaneous movements (Fig. D). The activity in the two limbs was not synchronous but one limb was usually leading the other with a rather stable lag (Fig. 1 B, cf. also Fig. 3 in Grillner 1973 and Cohen 1979). Some cats could maintain long periods of stable "hindlimb gallop" while others performed only 5–6 steps and then reverted to alternation. The readiness to respond with "gallop" changed with time so that they could exhibit stable "gallop" at a very low speed at one time and somewhat later only at a much higher treadmill velocity. Long term changes were also observed.

II. *Locomotion on split belts*

1. *Effects on different parts of the stepcycle.* The duration of the stepcycles decreases at faster walking speeds (Forssberg et al. 1980, Fig. 2). From this it could be inferred that when two limbs of the same girdle walk at different speeds they should walk at different frequencies if the cycle duration was only determined by the walking speed. In Fig. 3

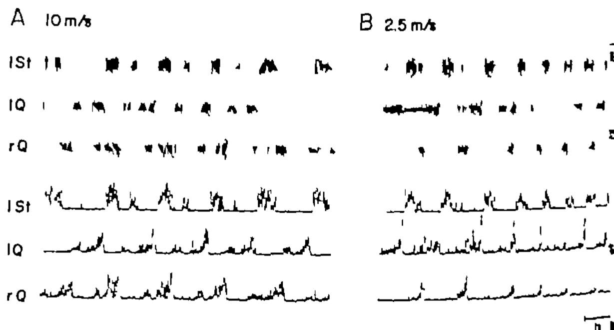


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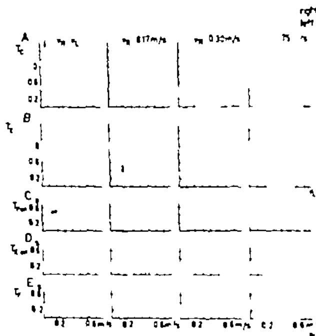


Fig. 4 Adjustments of the step cycle during locomotion on split belt. One belt was driven at constant speed (the right motor driven belt) and the other driven at various speeds. The speed of the right belt is given at the top of each column, the speed of the left is along the abscissa in each plot. In the column to the left the belts were "tied" together and driven at the same speed. A, B, C and D the durations of the step cycle (T), the support phase (T), the flexion phase (T_F) and the first extension phase (T_{E1}) of both limbs are plotted versus the speed of the left limb. The durations are calculated from the angular movement in the knee or ankle (A and B) or from the ankle (C and D). E the duration of the flexion phase of the hips (T) are plotted. The mean of the durations of the right limb is indicated by open circles and those of the left by filled circles (5–16). S.D. is indicated when it exceed the circle. Some data points are missing due to difficulties in determining some phases from the angular movement (especially in the ankle). (From Mira.)

...se were much shorter. To maintain the same rhythm either the step cycles might be proportionally changed, i.e. each phase would occupy the same proportion of the step cycle (see Kulagin & Shik ...), or one phase might be more influenced than others. From Table 1 it is seen that the proportion taken up by the support phase was changed although the speed of each belt was the same in the different conditions. The duration of the support phase had instead a tendency to preferentially be influenced by the belt speed. When the speed of the first limb (left) was increased (see Fig. 4B ... =0.30 m/s) the duration of the support phase was decreased and followed a similar curve as during normal locomotion. If the speed of the "fast limb" (right) was held constant (see Fig. 4B V =0.75 m/s) the duration of the support phase was only slightly shorter when the speed of the "slow limb" was decreased. In the latter the duration of the support phase increased when the speed decreased (Fig. 4B V =0.75 m/s) or decreased only slightly at constant speed when the "faster limb" walked faster (Fig. 4B V =0.17 m/s and 0.30 m/s). There could however be substantial deviations (see Table 1 and below).

As a consequence of the diverging influences from the two limbs during "split belt" locomotion *the duration of the support phase of the slow limb was always longer than that of the fast limb* (see Table 1 and Fig. 4B).

To maintain the same rhythm the differences in support phase had to be compensated for. Three different types of changes were recognized. (I) In the "fast limb" the swing phase was prolonged and

Table 1 *Modification of the step cycle during split belt locomotion in four spinal cats*

To the right the two limbs simultaneously walk at different speeds ("split belt") To the left the limbs walk at the same speed ("tied belts") The latter speeds are chosen to be the same as those of each split belt The duration of the step cycle (in 1–4) and the real and relative ((support phase/step cycle) ×100 %) duration of the support phase in 3–4 are compared with regard to the same limb in each condition Means and standard deviations are calculated in 3–4 and the data are compared by Students *t*-test $p<0.05$ = and $p<0.01$ = Note however that the variation of the left speed is not measured in the different conditions In Elsa ( ) with a dominance of the left limb the data on the split belt are taken from two different sessions first with the left limb walking on the slow belt and then with the left limb on the "fast belt

		Tied belts				Split belt		
		Speed (m/s)	Cycle dur (ms)	E_1+E (ms)	%	Cycle dur	E_1+E_1	%
1	Tiger	0 60	1 51 ±60			1 309± 61		
		0 90	988±52			1 314± 67		
	Elsa	0 75	1 149±11			1 109± 50	left } see Legend	
		1 00	732±43			876± 44	left }	
3	Liza	0 15	1 131±69	849± 75	75	996± 46	831±46	8[illegible]
		0 50	546±3	246± 4	46	989± 65	330±57*	3[illegible]
		0 15	1 131±69	849± 75	75	753±105	490+71	6[illegible]
		0 40	620±45	313± 53	50	75 ± 81	764±3[illegible]	5[illegible]
4	Mina	0 17	849±86	4_3±100	50	684± 61	480±99	70
		0 75	680± 4	279± 29	41	681± 45	13±19*	31



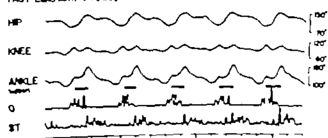


Fig 3 Locomotion of a chronic spinal cat on split belt in a 1 1 rhythm The angular movement of the hip knee and ankle and the synchronously recorded rectified and filtered EMGs of Q and St are shown for both limbs The support phases are indicated with bars. The belt on the right side was driven at 0.30 m/s and that of the left at 0 70 m/s. Angular calibrations are to the right of each curve and the time calibration is on the bottom in second (large bars) with 200 ms subdivisions (small bars) (From Mina)

the left limb was walking on a belt which was more than twice as fast as that of the right limb still the two limbs walked at the same rhythm Hence there are some spinal mechanisms to coordinate the cycles to equalize their duration In Table 1 the duration of the step cycles at different velocities during split belt walking is compared with that during locomotion at the same speed on tied belts Usually the cycle of the fast limb was prolonged and that of the slow shortened as for mesencephalic cats (Kulagin & Shik 1970) The degree of shortening and prolongation varied, however between the different cats and also between different trials in the same cat One of the limbs dominated usually and had a stronger influence on the rhythm In one cat (Tiger) the slow limb dominated slightly (irrespective of left or right) in another (Mina) the fast limb had the most powerful influence and determined the rhythm. In Elsa the left limb was dominant whether it was the slow or the fast limb and finally in Liza either the slow or the fast limb could have the largest influence

In Fig 3 there are striking differences between the muscular activity and the angular movement in the two limbs The extensor burst and the support phase of the slow limb were much longer than those of the fast limb and th burst and flexor

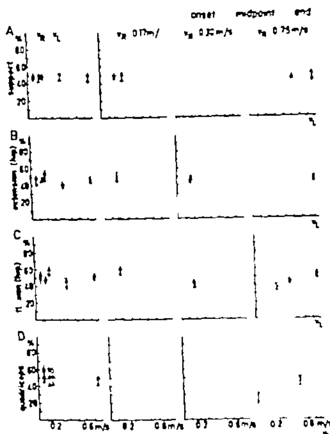


Fig. 6. Coordination between some events of the locomotor activity of the two limbs. The same experimental paradigm as in Fig. 4 is used. The speed of the right belt is above each column, the speed of the left is along the abscissa. The relative interval ((interval/cycleduration) · 100 %) from the onset (triangles) or midpoint (circles) of the left limb to the corresponding event of the right limb is plotted for: *A* the support phase, *B* the extension and *C* the flexion phase of the hip and for *D* the quadriceps activity. In *D* the relative interval between the termination of activity in the two limbs is also plotted. The mean and S.D. are indicated (n = 5–16). The relation of the movement of the knees and of the ankles changes in a similar way. (From Miller.)

(Fig. 6). Even if the onset of the different ... did change, it might have been expected that ... midpoints of e.g. the support phases should ... remained in strict alternation (circles in Fig. ...) so it should mean that whole phases would ... been reciprocally coupled. However this was ... the case (Fig. 6).

The onset of the extensor activity (Fig. 6D) ex... (Fig. 6B) and the placement of the feet (Fig. ...) were nevertheless all relatively close to 50% ... varied less than e.g. the midpoints or termina... (Fig. 6D). The relations between the onset of ... flexor muscles or the onset of flexion (Fig. 6C) ... varied substantially at the different speed combina-tions but in a linear way depending on the more constant extensor part and on the changed relative durations of the support phases (see above).

3. *Locomotion with different rhythms of the two limbs.* At extreme differences between the speed of the two belts the compensatory changes of the step-cycles (see sect. II. 1) were not sufficient to maintain a common rhythm of the two limbs. Instead of a prolonged waiting in the swing phase, the "fast" limb executed an extra step during the support phase of the "slow" limb (Fig. 7A). The occurrence of extra steps depended on the relative speed difference between the two belts but also on the leg assymmetries (see sect. I). At certain speed differ-

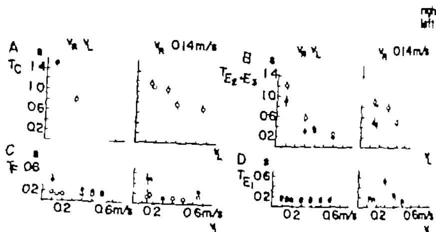


Fig 5 Adjustments during locomotion on split belts The same experimental paradigm as in Fig 4 is used The left part of each graph shows the situation when both belts are driven at the same speed In the right part the right belt is driven at a constant speed of 0 14 m/s and the left belt driven at various speeds along the abscissa The duration of *A* the stepcycle (T) *B* the support phase (T_{E_2} E_3) *C* the flexion phase (T) *D* the first extension phase (T_{E_1}) as determined from the angular movement of the ankles are plotted against the velocity of the left limb The mean and S D are plotted for both limbs (*n*=5–14) Open circles are from the right limb and filled circles from the left (From L1za)

the limb "waited" in the last part of flexion or in the first extension phase (Fig 3) (*ii*) In the slow limb" the swing phase was shortened usually due mainly to a reduction of the flexion phase (Fig 4 C) with only small changes in the first extension phase (Fig 4 D) (*iii*) In the slow limb" there could also be a substantial reduction of the support phase (Table 1 3) These three compensatory changes could be identified in all cats but their relative importance differed depending on whether the 'fast" or the slow limb dominated the rhythm (see above)

The influence from the fast limb to reduce the support phase of the slow limb (iii) would be opposite to that of the peripheral feedback from the "slow limb" which would act to maintain the duration of the support phase In Fig 5 one may recognize a shift in the balance of these conflicting influences At the highest and the third highest speed of the left (fast) limb the influence from the 'fast limb dominated with a shortening of the support phase of the "slow limb" (Fig 5B) and of the common step cycle duration (Fig 5 A) At the other speeds of the fast limb" the cycle duration and the support phase of the slow limb remained constant (Fig 5 A B) In the latter cases the "fast limb" had to adapt by waiting in flexion (Fig. 5 C 0 50 m/s) or first extension (Fig 5 D 0 30 m/s) with a long duration of the stepcycle (Fig. 5 A)

? *Coordination between the limbs* During strict alternating locomotion (walk or trot) with symmetric stepcycles the muscular activity and the movements in one limb will follow the same events of the other limb after exactly *half* a step cycle The events are thus shifted by 50% although there can be some variability around the mean (Stuart et al 1973 English 1979 cf Halbertsma et al 1976 In the present split belt situation the relative durations of the different parts of the step cycle are changed and thus there is no possibility for *all* events to remain 50% out of phase If some events should be more tightly linked they should maintain a fixed phase relationship independently of other changes Another mechanism would be a constant lag between two opposite events e g flexion in one limb and extension in the other No such constant lag was found and in fact it could not be a possible mechanism since the relative duration of the different phases changes as the speed of locomotion varies (Goslow et al 1973) Forssberg et al 1980

Fig 6, left column shows how the onset and the midpoint of different phases remain at approximately 50% when the two limbs walk at the same speed (see also legend Fig 6) In the 14 different combinations of speeds between the left and the right side shown in the three vertical columns to the right there is no indication that the onset of each support extension flexion or quadriceps EMG activity remain at one fixed ase relationship (the

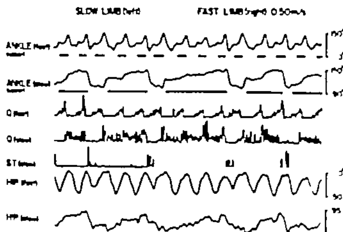


Fig 8 Relative coordination (–4 1) of chronic spinal cat walking on split belt The angular movement of the ankle and hip and the rectified and filtered EMGs are presented as in Fig 3 The velocity of the low belt ned bet een 0 06 and 0 11 m/s, that of the fast belt as 0 50 m/s (Note time and angular calibrations) (From Lura)

?) or during 1 or 3 1 gait the extensors of ...o sides co-contract during part of the step-

A reciprocal pparent depression of the ...activity was often seen as in the 2 1 steps of 7A, hen the extensor burst of the fast limb ...aller when it occurs during the extensor burst ... slow limb (cf also Fig 8) The a eraged ...th of the extensor activity of the slow l mb ... 7B do also show depression in the beginning ...e contralateral extensor burst In the slow walk ...ted belt in Fig 9A it is striking that the amplitude was much larger when no co-acti... occurred This reciprocal interaction was ...times very apparent, especially in the knee ...nsors, and at other times not obvious The ...onal basis is unknown. It may be a central ...n between parts of the generator network or ...ts on the motoneurones or premotor inter...rones It may also be of reflex origin although it ...ld be noted that the depression usually started ...re the foot was placed on ground

...ome signs of reciprocal effects between co-con...ting extensors and flexors were also seen. The ...nd St burst in Fig 9B coincides with a phasic ...ression of the Q-burst Such reciprocal relations ...e also been seen afte deafferentation in walking ...erebrate cats (Grillner & Zangger unpubl) and ...ld advocate a central origin of the effects

...n some instances, during the 1 gait there ...re small flexor bursts in the slow limb during the long extensor bursts (cf the 1st and the 4 th IP and TA bursts in Fig 7A) They may represent abor tive flexor burst depressed somehow by the mechanisms responsible for the prolongation of ex tensor burst

DISCUSSION

The pinal cord can no doubt coord nate the hindlimbs in two different modes in alternation a during walk or trot or in non-alternation as in a gallop (Fig Forssberg & Grillner 1973 cf. Miller & van der Meché 1976) The switch between them sudden and not gradual It may occur already at low speeds but the likelihood increases at high speeds There i however no necessary link be tween speed and type of coordination a an animal may sometimes witch at very low speed and some minutes later only at a higher Either the velocity or duration of the extension or the duration of the support phase can probably be a critical factor de termining the switch between the different gaits. However they can thus not be the only factors.

That the two legs are controlled by two separate entral spinal networks, one for each l mb (on Holst 1939 Shik & Orlovsky 1965 Kulagin & Shik 1970 Grillner & Zangger 1979) is a view that has been further corroborated in the present study as one limb could be made to walk one step when the other performed two or even four steps (Figs 7 and 8) Clearly this shift could be achieved by spinal

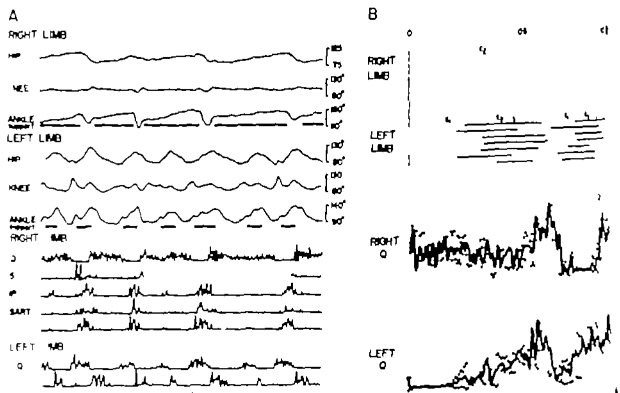


Fig 7 Relative coordination (1) during split belt locomotion *A* The angular movement in hip knee and ankle together with the period of support (bars) for the right and left limb are shown at the top and the simultaneously recorded EMGs (rectified and filtered) are presented below The velocity of the right belt varied between 0 and ... m/s and that of the left was 0 90 m/ Detail as in Fig 3 Note time and angular calibration *B* Schematic representations of the movements normalized to the stepcycles is shown at the top and the normalized and averaged Q-activity shown below during a stable 2 1 rhythm The support phase (E_2+E) is indicated with a bar the swing phase subdivided in the flexion (F) and first extension (E) phase with a dot (determined from the angular movement of the ankle) The rectified and filtered EMGs of the right and left Q (except for the second and sixth right stepcycle) averaged after normalization The mean is indicated as a continous line and the standard deviation as dotted lines cycle starts with the onset of the support of the right limb (From Lars)

ences periods of stable 1 coordination occurred (Fig 7 B) and with larger differences 3 1 and even 4 1 rhythms could be obtained (Fig 8)

The first extension phases and the onset of extensor activity of the two limbs never appeared to overlap (see Figs 7 A B and 8) If one limb approached ground to make contact the other waited (if in this part of the stepcycle) until the contact and proceeded only thereafter with its own F The support phase of the fast limb" was often completed before the "slow limb made contact (Fig 7 B see legend Fig 8) During one stepcycle of the slow limb there was always a multiple of steps in the fast limb This was probably due to the fact that the "fast limb" executed one step in relation to the swing phase of the slow limb and 1 to 3 steps during the support phase

The duration and the structure of the stepcycles of the fast limb" differed markedly depending on the phase of activity in the other limb During the 2 1 rhythm the steps occurring during the contralateral swing phase were much shorter than those during the support phase with a reduction of all the elements of the cycle (Fig. 7B) At higher rhythms (i e 3 1 and 4 1) the overall frequency in the fast limb was higher than during normal treadmill locomotion at the same speed The stepcycles that occurred during the swing phase (starting with flexion at the very end of the support phase) was the shortest as during 1 rhythm (Fig 8) The shortening was due to a short and prompt flexion and a prompt F_1 while the support phase was relatively long (Fig 8 see ankle) In other phases of the contralateral stepcycle i e the support phase the flexion was slower the first extension phase longer and the support phase relatively shorter

4 *Reciprocal action during slow walk*

(1), the "fast limb" performs shorter stepcy than during normal conditions. In spinal walk its one limb could be stopped with maintained ... activity while the other continued to walk ... at a higher rate (Grillner & Rossignol 1978 also Grillner & Zangger 1979). A position de... crossed effect on the rhythm does also ... under these conditions. If the stopped limb is in a flexed position the walking movement on contralateral side will be rather feeble but if the ... is extended to a point close to that in which ... normally is initiated, the walking move... will become vigorous (Forssberg, Grillner & ...ignol unpubl.). The duration of the support ... during locomotion on split belt as well as amplitude of the leg movement are at most ... close to that, which would be observed ... walking with "tied belts". At extreme situa... (e.g. at the transition to 1) this strict relation ... be modified and the amplitude of the leg movements changed. These results clearly allow ... conclusion that the duration of the support ... is controlled not exclusively but to a very ... degree by peripheral signals (Pearsson & ... 1976, Grillner & Rossignol 1978, An... et al. 1978) from the moving limb. The ...pheral input from each limb thus tends to sepa... the rhythms of two limbs that are not walking ... same speed. There occur however compen...ry changes of the stepcycles that at moderate ... differences lock the limbs in a common ... On the "fast side" the first part of the ... is comparatively unchanged but a prolonga... occurs in late F or in the first half of E_1. The ... may even occasionally "wait" at the transition ... E_1 and E_2. On the "slow side" there is ...ally a shortening of the flexion phase and ... also a reduction of the support phase. ... latter effect is however opposite to the ...pheral influence on the support phase. The ...ges therefore preferentially occur during the ... phase and then especially in the interval prior ... placement. After an unpredicted disturbance ... movement in one limb which elicited a com...satory reaction that prolonged or reduced the ...tion of the stepcycle a regular walk was still ...ablished by readjustments in the following swing ... (Forssberg et al. 1977 cf. Forssberg 1979). I... ... cats with one limb partly denervated and ... for recording, substantial readjustments ... also occur during the extensor bursts ...

(Duysens 1977). In this situation however the phasic peripheral feed back was severely disturbed (see however also Duysens & Stein 1978). Hence the duration of the different phases are all modifiable at a given stepcycle duration. The structure of the stepcycle is thus highly modifiable. This is at variance with Kulagin & Shik (1970) who concluded that the structure of the stepcycle was determined by the speed of the belt and kept constant also during the split belt situation in contrast to the duration of the whole stepcycle which was allowed to vary. They did actually encounter changes of 10 and sometimes up to 30% in their material. They measured however the extension and flexion of the hip which may have rendered the disclosure of these effects difficult.

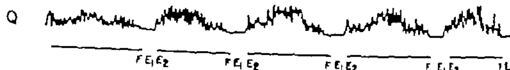
The fact that the different parts of the stepcycle are modifiable becomes even clearer when one considers the 1 (or 3 1) coordination when the two steps on the fast belt are assymetric despite the fact that the belt speed is the same. This can in fact be seen also in one illustration of Kulagin & Shik (1970; Fig. 3) in which the step of the "fast limb" occurring during the swing phase of the "slow" has a faster flexion compared to steps occurring at the support phases of the slow limb. Thus the structure of a step depends on the prevailing phase of locomotor activity in the other limb. Hence crossed signals do not only control the coordination between the two sides but may actually also influence the cycle duration, the duration of the different phases, i.e. the structure of the cycle, as well as the step length on the contralateral side.

Interlimb coordination. The coordination between the two limbs is not achieved by a fixed lag between two separate events in the respective stepcycles (see Result sect. II 2). It is more likely that central signals related to the muscle activity in one or several phases of the step on one side project to the other side too. These signals may take form of "efference copies" (Stein 1974 and 1976 see Introduction). Undoubtedly interaction of some sort occurs between the different elements of the two central generator networks at the spinal level (Grillner & Zangger 1979). As the basic design of the neuronal network that constitutes the generator is unknown it is impossible at present to hypothezise about what type of neuronal interactions might occur between the two generators. On the other hand it is possible to define how the two networks actually do interact.

A 0.07 m/s

right

Q



left

Q



B 0.70 m/s

Q

ST



Fig 9 Reciprocal actions *A* The rectified and filtered EMGs of the right and left Q during very slow walking are together with the schematic movement of the ankle The bars indicate the support phase the dots the onset of the extension phases (E) *B* The rectified and filtered EMGs of Q and St of the same limb are shown together w schematic movements of the ankle Note differences in time calibration (From Mina)

mechanisms without influence from supraspinal structures

The neuronal mechanism allowing the switch between "gallop" and alternate modes of coordination has been depicted as depending on two alternative pathways One might provide reciprocal inhibition between congruent parts of the two generators e g the flexor parts could mutually inhibit each other resulting in alternation (a similar effect would be achieved by excitation between extensors on one side and flexors on the other) Another might mediate mutual excitation between corresponding elements of the two generators and thereby elicit the gallop type of coordination (see Halbertsma et al 1976 Grillner 1975) A control signal (e g descending or reflex) that caused a switch from one alternative pathway to the other could automatically switch the gait from one type to the other in a very simple way This general idea has so far not been tested experimentally

The 'split belt' situation

Effects on the stepcycle A readjustment of stepcycles of the two sides are necessary wh animal walks along a curvature The experim with split belts mimick this situation and show spinal mechanisms are sufficient to induce the appropriate changes Moreover in the experim situation the differences can be taken much fu than during ordinary conditions and thereby properties of the spinal coordinating system well as of the organization of the locom generator

In decerebrate cats the "slow and the limb will at moderate speed differences ha cycle duration that is intermediate between th walking at either the slow or the fast speed (K & Shik 1970) This may be true also in the sp (see Table 1) That means that the activity of limb influences the locom tor activity in the At different rhythms e limbs (i e

II, the "fast limb" performs shorter stepcy than during normal conditions. In spinal walk its one limb could be stopped with maintained motor activity while the other continued to walk, even at a higher rate (Grillner & Rossignol 1978 so Grillner & Zangger 1979). A position de- ent crossed effect on the rhythm does also under these conditions. If the stopped limb is in a flexed position the walking movements on contralateral side will be rather feeble, but if the is extended to a point close to that in which normally is initiated the walking move- will become vigorous (Forssberg, Grillner & unpubl.). The duration of the support during locomotion on "split belts" as well a amplitude of the leg movements are at most is close to that, which would be observed walking with "tied belt". At extreme situa- (e.g. at the transition to 2 : 1) this strict relation be modified and the amplitude of the leg ments changed. These results clearly allow conclusion that the duration of the support is controlled not exclusively but to a very ficant degree by peripheral signals (Pearsson & ens 1976, Grillner & Rossignol 1978, An- son et al. 1978) from the moving limb. The pheral input from each limb thus tends to sepa- the rhythms of two limbs that are not walking same speed. There occur however compen- ry changes of the stepcycles that at moderate differences lock the limbs in a common . On the "fast side" the first part of the on is comparatively unchanged but a prolonga- occurs in late F or in the first half of E_1. The may even occasionally "wait" at the transition ween E_1 and E_4. On the "slow side" there tually a shortening of the flexion phase and sometimes also a reduction of the support phase. latter effect is however opposite to the pheral influence on the support phase. The ges therefore preferentially occur during the ng phase and then especially in the interval prior foot placement. After an unpredicted disturbance the movement in one limb which elicited com- satory reaction that prolonged or reduced the ation of the stepcycle, a regular walk was still ablished by readjustments in the following swing e (Forssberg et al. 1977 cf. Forssberg 1979). In walking cats, with one limb partly denervated and planted for recording, substantial readjustments could also occur during the extensor bursts (Duysens 1977). In this situation, however, the phasic peripheral feed back was severely disturbed (see however also Duysens & Stein 1978). Hence the duration of the different phases are all modifi- able at a given stepcycle duration. The structure of the stepcycle is thus highly modifiable. This is at variance with Kulagin & Shik (1970) who concluded that the structure of the stepcycle was determined by the speed of the belt and kept constant also during the split belt situation in contrast to the dura- tion of the whole stepcycle which was allowed to vary. They did actually encounter changes of 10 and sometimes up to 30% in their material. They measured however the extension and flexion of the hip, which may have rendered the disclosure of these effects difficult.

The fact that the different parts of the stepcycle are modifiable becomes even clearer when one considers the 2 : 1 (or 3 : 1) coordination when the two steps on the fast belt are asymmetric despite the fact that the belt speed is the same. This can in fact be seen also in one illustration of Kulagin & Shik (1970: Fig. 3) in which the step of the "fast limb" occurring during the swing phase of the "slow" has a faster flexion compared to steps occurring at the support phases of the "slow limb". Thus the struc- ture of a step depends on the prevailing phase of locomotor activity in the other limb. Hence crossed signals do not only control the coordination between the two sides but may actually also influ- ence the cycle duration, the duration of the different phases, i.e. the structure of the cycle as well as the step length on the contralateral side.

Interlimb coordination. The coordination be- tween the two limbs is not achieved by a fixed lag between two separate events in the respective step- cycles (see Result sect. II 2). It is more likely that central signals related to the muscle activity in one or several phases of the step on one side project to the other side too. These signals may take form of efference copies (Stein 1974 and 1976 see Intro- duction). Undoubtedly interaction of some sort oc- curs between the different elements of the two cen- tral generator network at the spinal level (Grillner & Zangger 1979). As the basic design of the neuro- nal network that constitutes the generator is un- known, it is impossible at present to hypothesize about what type of neuronal interactions might oc- cur between the two generators. On the other hand it is possible to define how the two networks ac- tually do interact.

It is important to realize that any peripheral stimulus that affects the activity of one generator can indirectly via the coordinating links influence the pattern of activity of the generator of the other side Crossed effects on the structure of the cycle have been demonstrated (Grillner & Zangger 1979) but it is not clear whether they are mediated in this indirect way or induced by direct crossed effects The fact that central mechanisms for interlimb coordination exists does of course not exclude the possibility that crossed peripheral factors may also contribute during locomotion (see Forssberg 1979)

The coordination in the split belt situation is apparently not a random at any combination of speeds. In both 1 1 or 2 1 the two hindlimbs are coordinated in a seemingly stable way so stable even that the two stepcycles of the "fast limb in a 2 1 gait are asymmetric in a characteristic way It may be relevant that the best fit to strict alternation between the two limbs are the periods between onset of hip extension and foot contact which constitute the last part of E_1 (see Fig. 6) Furthermore it is noteworthy that the occurrence of E in one limb seems to exclude its occurrence in the other limb also at 2 1 or 3–4 1 gaits The initial part of E may be passive in the knee and ankle but only after the termination of the flexor activity (ankle) In contrast the hip extension is actively controlled as well as other features including the early cocontraction of foot flexors and extensors (Engberg 1964 Forssberg et al 1980) Adjustments of the step cycle may be made during this period (E) (see above) For instance the ground contact must be smooth regardless of the quality of the terrain This phase could also be involved in the coordinating control Reciprocal inhibition between symmetrical events may thus be a possible contributor during alternate gaits Other factors that may contribute could be a mutual net facilitation between neurones generating the same or different phases on the two sides A facilitation from the neurones controlling flexion on one side (slow) to the flexion and extension networks on the other side (fast) could explain the interaction resulting in the quick but forceful step occurring during the swing phase of the "slow limb (see Figs 7 and 8)

It may be expected that not one but several different mechanisms are operating to ascertain the interlimb coordination Each mechanism may "push or pull" to promote its particular coordinating feature They will constitute at least two sets of mechanisms one for the gallop mode and one for alternation (see above)

CONCLUSION

The chronic spinal cat can coordinate its hindlimbs in a variety of ways not only in alternation and gallop modes but also in adjusting the relative duration of support and swing phases to meet specific requirements such as during turning An interesting implication of the present study is that the brain does not necessarily need to concern itself with adjustments of the hindlimbs when the animal is turning It would actually be sufficient for the front part to turn and the hindlimbs would by the spinal reflex machinery make the appropriate changes (cf Andersson et al 1978 Grillner 1979, 1980) A change-over from gallop to alternation might be achieved by a switch from one set of alternative spinal pathway to another There is however no reason to exlude additional supraspinal control mechanisms

In the stepcycle of a limb the duration of the support phase is determined primarily by peripheral inflow the first part of the flexion is rather constant whereas most of the necessary timing adjustments occur during F_1 or the last part of the flexion phase It must be emphasized however that no element in the stepcycle is constant—all modifiable

The excellent help of Mrs Margret S[illegible]berg, Miss [illegible] and Inger Klingenbrant is gratefully acknowledged [illegible] work was supported by the Swedish Medical Research Council project no 14X 3026. J H was supported in part by the Delft University of Technology The Netherlands S R was supported by the Canadian Medical Research Council

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Physiol Scand 1980, 108 297 300

lease of gastrin and somatostatin into the stric lumen of healthy subjects and patients th duodenal ulcer and achlorhydria

WISÉN KERSTIN UVNÄS-WALLENSTEN SUAD EFENDIĆ and CATJA JOHANSSON

WISÉN O UVNÄS-WALLENSTEN K EFENDIĆ S & JOHANSSON C Release of gastrin and somatostatin into the gastric lumen of healthy subjects and patients with duodenal ulcer and achlorhydria Acta Physiol Scand 1980 108 297 300. Received July 1979 ISSN 0001-6772 Gastrointestinal Unit Department of Medicine Department of Endocrinology Karolinska Hospital and Department of Pharmacology Karolinska Institute Stockholm Sweden

Gastrin and somatostatin were measured in alkaline gastric insufflates in normal subjects patients with duodenal ulcer disease in quiescent state and in patients with achlorhydria. Both peptides were released into the lumen. The gastrin-somatostatin ratio (G/S) in healthy subjects was approximately three. Duodenal ulcer patients had significantly lower G/S ratio due to lower gastrin and higher somatostatin levels whereas in patients with achlorhydria the G/S ratio did not differ from normal subjects

...rin and somatostatin have been demonstrated ...he G- and D-cells of the gastric antrum These ...mones are released into the portal vein as well a ...the gastric lumen. Thus gastrin has been disco- ...ed in the gastric lumen in cat (Uvnäs-Wallensten ...1976) and dog (Rehfeld et al 1978) and man ...Green et al. 1978) and verified as gastrin 17 ...the cat and as gastrin 17 and 34 (Rehfeld et al. ...) in the dog A pH-dependent release of gastrin ...somatostatin into the gastric lumen of cats was ...wn following vagal stimulation (Uvnäs-Wal- ...sten et al 1977) with relative dominance of ...somatostatin during perfusion at pH 1 and of gas- ...at pH 4–5 It has been suggested that the two ...tides exert paracrine effects and interact locally ...modulate gastric acid secretion from the parietal ...ls (Pearse et al 1977). At pH 7 4 both gastrin and ...somatostatin are released into the perfusate prob- ...ly due to a direct effect of the high pH on the ...docrine cells (Uvnäs Wallensten unpublished ...ta) In the present study we have determined ...vels of gastrin and somatostatin in the gastric lu- ...en in healthy subjects and in patients with ...odenal ulcer disease and achlorhydria.

METHODS

Gastrin and somatostatin were determined in gastric aspirates from 14 healthy subjects (21 to 34 years old, mean 24 years) without history of gastrointestinal disease in 10 achlorhydric patients and in 10 patients with duodenal ulcer disease (28 to 54 years old mean 46 years) The diagnosis of duodenal ulcer had been confirmed by X-ray and/or endoscopic examination. None had demonstrated complications such as perforation massive hemorrhage or secondary gastric ulcer On an average 14 years had elapsed since the first symptom and the course of the disease had been relapsing At the time of the present study no patient had symptoms or signs of an active ulcer Achlorhydria had been established in 10 patients, aged 29 to 72 years (mean 58) by examining the gastric secretory response to s.c. pentagastrin, 6 µg kg (Peptavlon ICI England) The mean fasting serum gastrin level in the patients was 393±86 pg

Informed consent was obtained from each subject and the study had been approved by the Ethical Committee of the Karolinska Hospital Stockholm

No medication was allowed 24 h prior to the study After an overnight fast the subjects were intubated with a double lumen gastric tube composed of a Ryle tube 14 FG (Portex England) used for aspiration of gastric contents and another Ryle tube 10 FG ending 6 cm proximally to the first one prepared with additional openings at the tip and used for instillation of sodium bicarbonate

Table 1 *Ratio between concentrations (pm l⁻¹) of gastrin and somatostatin in aspirates 1 and 2 Mean±S E*

	Subjects no	G/S ratio in aspirate 1 and		
		1	2	(1+ 2)/2
Controls	14	3 2±0 8	2 7±0 7	3 0±0 7
Duodenal ulcer	10	0 7±0.3	0 6±0.3	0 6±0 3
Achlorhydria	10	2.5±0 6	.8±1 6	7±1 1

P-level<0 02 for difference from controls
P level<0 01 for difference from controls.

With the subject on his left side 250 ml isotonic sodium bicarbonate pH 8 (Sodium bicarbonate 0 167 M ACO Sweden) was instilled into the stomach during 2 min A 120 ml sample of the gastric contents was withdrawn at 6 min after initiation of the instillation (aspirate 1) and was replaced by an identical volume of the solution After another 6 min a second 120 ml sample was withdrawn (aspirate 2)

100 ml of each aspirate was immediately adjusted to pH 8 with 1 M NaOH and boiled for 15 min The sample thus reduced to 1/5 of the original volume was rapidly frozen and stored at −20°C until analysed for hormone concentrations.

Concentrations of gastrin were determined by radioimmunoassay (Nilsson 1975) using antiserum 604 kindly supplied by professor J Rehfeld University of Aarhus Denmark Somatostatin was determined by radioimmunoassay using own antibodies (Efendic et al 1978) Concentrations of gastrin and somatostatin were expressed in molar units in calculating ratios between hormone concentrations in the aspirates

The remaining 20 ml of the aspirate was used for analyses of electrolyte concentrations Concentrations of sodium were determined by flame photometry and of chlorides by coulometric titration Concentrations of bicarbonate were determined after adjustment of the sample to pH 10 with NaOH Backtitration was made with 0 03 M NaOH to endpoint pH 10 after addition of [illegible] H_2SO_4 and boiling

Wilcoxon s nonparametric rank test was used to test significance Results are given as mean±S E

RESULTS

The ratios between concentrations of gastrin and somatostatin in the aspirates were not different in the first compared to the second aspirate in either of the groups studied Healthy subjects released 3 0±0 7 times more gastrin than somatostatin into the gastric lumen (Table 1) The G/S ratio was not different in patients with achlorhydria but was significantly lower in duodenal ulcer patients ($P<0\ 01$)

The degree of volume reduction by boiling was similar in all series of aspirates (Table 2). Electrolyte concentrations in unboiled samples (Table 2) showed similar degrees of dilution and neutralization in the two control aspirates and the first aspirate from the duodenal ulcer group. Hormone concentrations as expressed in pm l⁻¹ in unboiled aspirates could thus be compared between healthy controls and first aspirate from patients with duodenal ulcer although values from all aspirates are presented in Table 3 The low G/S ratio in the duodenal ulcer group was due to reduced levels of gastrin ($P<$[illegible]) and elevated levels of somatostatin ($P<0\ 05$)

DISCUSSION

Gastrin and somatostatin were released into the lumen of the intact human stomach after instillation of a sodium bicarbonate solution Healthy [illegible]

Table 2 *Concentrations of electrolytes in unboiled aspirates from healthy controls (n=14) patients with duodenal ulcer (n=10) and achlorhydria (n=10)*

Last paragraph shows that the volume reduction by boiling prior to hormone analyses was similar in all series

Subjects	Asp	Na (mmol l⁻¹)	Cl (mmol l⁻¹)	HCO_3 (mmol l⁻¹)	Volume reduction (%)
Controls	1	137±7	2±4	1 1± 8	80±1
	2	139±8	20±3	127±11	72±4
Duodenal ulcer	1	139±5	4±4	117± 8	79±1
		131±7	4 +9	80+14	82±1
Achlorhydria	1	142±10	8±	140± 7	80±1
		154± 5	8±1	139± 6	79±

e 3 Concentrations of gastrin and somatosta ... expressed in pm l in a ... led gastric a pl

...entrations are comparable only bet een control ...tes and first aspirate in the duodenal ulcer group ... the degrees of dilution and neutralization differ from ...ch in the second aspirate from duodenal ulcer pa...ted in both aspirates from patients with achlorhy

...ts		Asp	Gastrin	Somato-statin
...ols	14	1	15 4	7 1
			18 4	7 1
...enal ulcer	10	1	5 3	15±8
		2	5 3	8 1
...rhydria	10	1	4 6	10 1
		2	18 5	13±3

...d <0 05 for difference from control aspirates

...n average released 3 times more gastrin than ...ostatin, and this was true also for achlorhy... patients On the other hand in the peptic ulcer ...p, the proportion between hormone levels wa... 1 The advantage of using an alkaline buffer in ...ntact human stomach is that the action of gas... pepsin is prevented However proteolytic ...mes may be present in the aspirates from regur...ed duodenal contents and have t be inacti...d. By boiling the aspirates immediately upon ...drawal destruction of the hormones and ar...ts in the radioimmunoassay were avoided ...ers et al 1976).

...r comparison of hormone concentrations in the ...t aspirate from healthy subjects and duodenal ...er patients is justified because of the similar elec...yte concentrations n the aspirates indicating ...ame degree of dilution and neutralization The ...n finding obtained was that reduced concentra...s of gastrin and elevated concentrations of ...atostatin contributed to the low G/S ratio in the ...denal patients

The present finding of low gastrin concentrations ...gastric aspirates in the ulcer patients is in line ...h the finding of decreased basal serum gastrin i... ...ch patients (Korman et al 1971 Trudeau et al ...70) However others have failed to show any sig...ficant difference in basal gastrin between sera ...m patients with duodenal ulcer and healthy con...ls (Walsh et al 1975). Polak et al (1976) found no differences in the number of G- and D- cells be tween healthy subjects and the majority of duodenal ulcer patient

According to Chayvialle the antral content of gastrin were not different in duodenal ulcer patient compared to healthy controls but the patients were found to ha e a 40% reduction of their somatostatin content (Chayvialle et al 1978) The apparent dis crepancies between these data when compared to each other and to the present result might be due to the use of different procedures and assay Further more the number of hormone producing cells or the hormone contents in the antral wall may not neccessarily reflect the hormone secreting capacity In addition the mechanism by which the release of gastrin and somatostatin is stimulated by alkaline buffer has not yet been identified

Another difference between studies is that the examined patient represent different stages of the peptic ulcer d sease Thus all patient examined by Chay ialle et al had ymptoms of an active ulcer and an endoscopically erified duodenal ulcer with mild or marked inflammation of the antral mucosa In contrast the present patient were examined in a symptom-free interval after a long history of un complicated relapsing duodenal ulcer disease

Should the hypothesis of a paracrine interaction of ntral gastrin and somatostatin (Pearse et al 1977) be correct then the present data may well be interpreted n terms of a long standing adaptation resulting in a dominance of somatostatin over gas trin

The poss bility to recover gastrointestinal pep tides i gastric and ntestinal contents may provide a useful tool for studies of pathophysiological mechanism in diseases of the gastrointestinal tract

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[F]unctional properties of neurones in the posterior part [of] area 7 in awake monkey

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The posterolateral part of the parietal association cortex (area 7 of Brodmann) was investigated in three awake behaving macaque monkey using transdural microelectrode recording technique Only one of the 114 cells isolated remained unidentified Of the cells 79% responded to somesthetic (39%) or visual (23%) or both somesthetic and visual (17%) stimulation. The somesthetic recepti fields were large covering for instance the whole arm or hand Most receptive fields were on the upper extremities Half of the somesthetically drivable cells had bilateral receptive fields. The visually drivable cells responded only to stimuli moving in a certain direction The effective stimuli for most of the cells responding both to visual and somatosensory stimulation were touching of the skin and visual stimuli moving towards the cutaneous receptive field Of the cell 20% were active only during the monkey own movements most often during grasping and manipulation with fingers The results indicate that the area studied is specialized in the control of hand movement e g grasping, grooming and somesthetic recognition of the forms of objects

Key words Parietal association cortex area 7 microelectrode recording

[In] their pioneering study of cellular function in [area] 7 Hyvärinen & Poranen (1974) recorded neurones that were activated by visual and somesthetic [stimu]li Mountcastle et al. (1975) and Lynch et al (1977) who recorded the medial parts of area 7 [conclud]ed that cellular activity in this area was mainly [relat]ed to the control of eye movements i.e the [cells] were active only during the movements of the [eye]s or they responded to visual stimuli Studies in [the] anterolateral part of area 7 showed that here [cellu]lar activity was related to the control of face [and] arm movements (Leinonen et al 1979 Leinonen & Nyman 1979) The purpose of this study was [to] explore the functional properties of the posterolateral part of area 7 medial to the end of the Sylvian [fis]sure which was not included in the previous [stu]dies

[The] neurones investigated responded to visual and [som]esthetic stimuli or discharged only during the [mon]key own movements. The activity of most [ne]urones was related to the control of hand move[me]nts, e g grasping and manipulation. The results of this study provide evidence for the concept that area 7 is intra-areally differentiated for the control of movements of different parts of the body

MATERIAL AND METHODS

Recordings were made in four hemispheres of two adult female and one juvenile male Macaca speciosa-monkeys weighing 6–8 kg Experiments on each monkey lasted 2–8 weeks. During the daily experiments of 3–5 h the monkey sat in primate chair with its head immobilized with halo attached to the skull The monkey could move its arms and legs The arm movements observed during the recordings were usually triggered by offering a raisin to the monkey The movements consisted of the following sequence: reaching, getting hold of and bringing the raisin to the mouth In order to relate the cellular activity to the arm movements sample device was constructed I the device raisin that the monkey was reaching for was placed in hol on board When the monkey finger was within the distance of 0.5–1 mm from the raisin photocell attached to the board above the raisin was activated By observing the monkey behaviou and using this apparatus we could determine whether the discharges of a

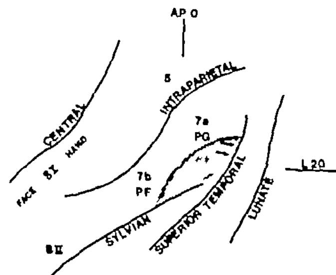


Fig. 1 The sulci of the parietal lobe and the recording site (hatched). The stereotaxic planes AP 0 and L 20 are indicated at the margins. The area recorded extend into the anterior wall of the superior temporal sulcus (arrows).

cell were related to (1) reaching and grasping for ( ) manipulation or (3) bringing the raising to the mouth. Horizontal and vertical components of eye movements were recorded with electrodes implanted into the skull around the eyes. Cellular activity signals from ocular electrodes timing signals of stimuli and verbal comment were stored on tape. The method of recording has been described in detail earlier (Hyvarinen & Poranen 1974, Leinonen et al. 1979).

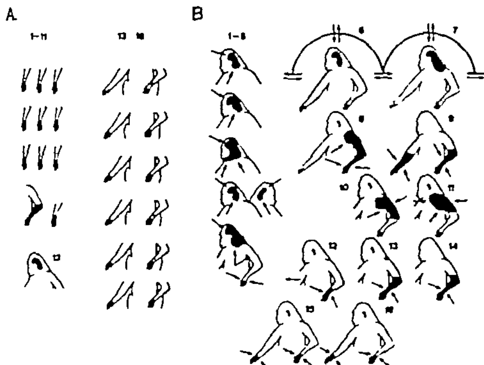
During one penetration –8 neurones were usually isolated. After a neurone was examined the electrode was brought 0.1–0 mm deeper. Often the neurones had some "spontaneous" activity which revealed their existence. The spontaneous activity here means that the neurones discharged at a low irregular rate when the monkey looked at people moving and talking around him and when the monkey occasionally moved the eyes and limbs. Sometimes however only a certain stimulation or a certain motor activity evoked by the investigator revealed a new neurone. The cellular responses were observed on an oscilloscope screen. The cellular activity was also fed through an audio monitor to a loudspeaker, the discharges were thus audible all the time. We noticed regularly that a group of stimuli which were similar in spatiotemporal appearance or other information content could activate the same cell in varying degrees. Quantitative estimation of the differences was not however possible because of the nonstandardized stimuli. Because we did not use histogram technique only clear and strong responses could be observed and analyzed. The properties of each neurone isolated were studied by presenting different stimuli and observing the monkey behaviour. Following stimuli were used: light touch, pressure on the skin, palpation and tapping of muscle bellies, rotation of joints, various moving and stationary visual stimuli (e.g. the experimenter's hand moving in different directions, ral ins bananas and novel small objects in front of the monkey).

All penetrations were made using the coordinate system of the Evarts microdrive. A three dimensional ... map of the target was obtained during recordings. The target areas in two hemispheres of the two female monkeys were macroscopically and histologically investigated. After the monkey was killed several guiding electrodes were inserted into the target area. The location of all penetrations were then studied in reference to the coordinates of these guiding electrodes. With this method each penetration could be located on the cortex with accuracy of about 1 mm. Near the sulci functional criteria were also used for the identification of the location of penetration. These criteria included (1) distance of the neurone from the cortical surface (from the first burst of activity recorded during the penetration) ( ) thickness of the white substance penetrated before re-entering the cortex within a sulcus (3) location of the sulci on the functional map obtained during the recordings. Using the marking electrodes the functional maps could be compared with the histological maps obtained from the brains anatomically examined. In one monkey ... still alive the penetrations were located using stereotaxic coordinates and functional criteria only. This method of localization of the target was considered to be sufficient for the purpose of this study.

The part of area 7 that was examined lies on the hemispheric surface and in the anterior wall of the superior temporal sulcus (Fig. 1). The superior temporal sulcus has been histologically examined by Seltzer & Pandya (1978). They have defined four different subareas within the anterior wall: areas PG, PGa, TAa and TPO. Area PG ... 7a of Vogt & Vogt 1919) extends only a short distance into the sulcus. Most of our cells were isolated in the hemispheric surface and only a few neurones were found in the superior temporal sulcus.

Table 1. Classification of the cells isolated in the posterolateral part of area 7 according to the ... of stimuli or type of behaviour related to the cellular activity

	No. of cells	[illegible]
Cells responding to sensory stimuli	90	[illegible]
Somesthetic	44	[illegible]
—touch or pressure on the skin	18	[illegible]
—palpation of muscles or rotation of joint	26	[illegible]
Visual	26	[illegible]
Visual and somatic	20	[illegible]
Cells active only during the monkey's own movement	[illegible]	[illegible]
Reaching	[illegible]	[illegible]
Grasping and manipulation	[illegible]	[illegible]
Not identified	1	[illegible]
Total	114	[illegible]



2. Receptive fields of the cells which responded only to touching or compressing of the skin (A) and of the cell which responded both to touching (or compressing) of the skin and to visual stimuli (B). Arrow indicate directions of movement of visual stimuli. Cells No. 6 and 7 responded to visual stimuli moving in the periphery of the visual field

RESULTS

114 cells isolated and studied were classified according to their responsiveness to stimulation of different sensory organs or according to the type of movement which correlated with the cellular activity (Table 1). Only one cell remained unidentified as its activity could not be influenced. Of the cell ... were active only during the monkey's own movements and 79% responded to some of the stimuli used, somatosensory or visual. The different classes of cells are reviewed below

Cells responding to touch or pressure of the skin

Fifteen cells responded only to cutaneous stimulation. Seventeen of them had a receptive field on the hands or arms (Fig. 2A). Usually the cells responded in an on, off or on–off fashion to compression of the fingers or hands, only one cell was responsive to light touch on the skin and blowing into the hair. The cells that had a receptive field on the fingers were active during the monkey's own hand movement, especially during grooming when the monkey pulled a hair between the thumb and forefinger and/or when it released the hair

Cells responding to palpation of muscles or rotation of joint

26 cells responded to palpation of a muscle belly in the arm (6 cells) or shoulder (? cells) or they were active during rapid or forced dorsal flexion of the wrist, fingers (9 cells) or ankle (3 cells). Four cells responded to palpation of the calf. The cells that had a receptive field on the arm were active when the monkey was grasping for or manipulating an object with its hand. Fig. 3 presents properties of a cell which was activated by tapping and squeezing of the ulnar muscle bellies. The cell discharged also during the monkey's own movements. It had no spontaneous activity

Cells responding only to visual stimuli

Twenty-six cells were driven only by visual stimuli. They were all isolated near or in the anterior wall

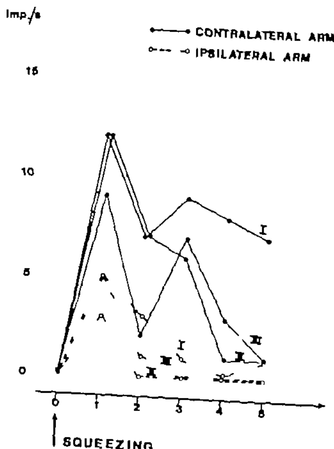


Fig 3 Firing rate of a cell during *the first 5 s of three* successive squeezings of the ulnar muscle belly *(I II III* interstimulus interval 20 s) The responses disappeared in 5–10 s after beginning of the squeezing

of the superior temporal sulcus *All these cells were* activated only by *moving stimuli 10* cells by stimuli moving in a tangential direction upwards downwards left or right 3 cells by rotating *objects* 2 cells by stimuli moving from the periphery of the visual field toward the centre of it 8 cells by objects that approached or were near the contralateral temple 3 cells by objects moving near the contralateral side of the head

Ten of the visually drivable cells were active when the stimulus moved in the periphery of the visual field 15 cells responded to stimuli moving in the central visual field The activity was not related to eye movements this was examined by making the monkey gaze at something else during the presentation of the relevant stimulus or by analyzing from tape the temporal relationship between the cellular responses and eye movements

Cells responsive both to visual and somesthetic stimuli

Sixteen cells responded to touching, pressing or streching of the skin or palpation of a muscle and to visual stimuli approaching the somesthetic receptive field or moving in the periphery of the visual field (Fig 2B) The receptive fields were on the hands or arms (8 cells) on the shoulder (4 cells) or on the temple (4 cells) Some cells were responsive to light touch on the skin and blowing on the hair some only to compression of the skin that caused deformation The cells that had a receptive field on the arm or hand responded to visual stimuli approaching the receptive field the visual stimuli were effective in both halves of the visual field Because of the head fixation the effective visual stimuli moving towards a receptive field on the shoulder always moved in the half of the visual field ipsilateral to the somesthetic receptive field Fig 4A shows responses of a cell to the investigator s hand approaching the contralateral temple (within 30 cm) During the withdrawal of the hand there is no activity A stationary visual stimulus near the cutaneous receptive field resulted in intermittent activity The activity stopped if the monkey s attention was drawn elsewhere with an interesting visual stimulus In Fig. 4B the same cell responds, when the monkey s eyes are covered

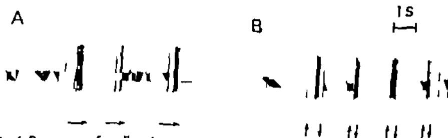
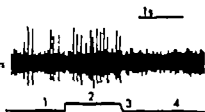


Fig 4 Responses of a cell to the experimenter's hand approaching (→) the contralateral temple (A) and to touching (↑↓) of the temple when the monkey s eyes were covered (B)



1 Activity of cell during reaching (1) digging — from hole (2), upward deflection of the DC potential when the monkey got hold of the rai the activity and there was no activity during the bringing of it to the mouth (3) When the monkey arms ere ... was no activity (4)

...ching of the contralateral temple The ef... visual stimuli usually caused no noticeable ...e contraction, not even the blinking reflex. ... the responses were not related to any d s... ...le muscle contraction.

...discharging only during the monkey ...movements

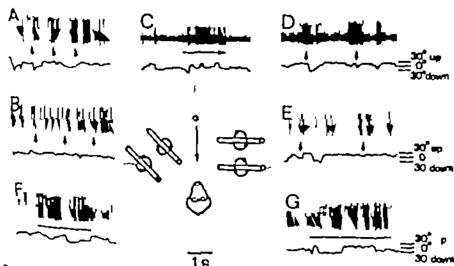
... ere active only during the monkey own ...ments, 3 cells during reaching and 19 during ...ing and manipulation with hands and one dur... ...nipulation with toes All neurones except one ...activated by movements of both sides of the body Fig. 5 shows discharges of a cell during reac... ing for and digging a raisin from a hole As soon as the monkey got hold of the raisin the activity stopped although the flexion of the fingers still continued

Cell d scharging both during manipulation ...th fingers (es covered) and during ...sual stimulation

Two cells responded to object rotating in the visual field and were also active during manipulation (Fig 6), two cell discharged when the monkey gra ped an object with it eyes closed or when the monkey saw the investigator grasp an object (Fig 7) The intensity of the response depended on the interest the object seemed to evoke in the monkey

Laterali ty f the ells

One half of the cells solated in area 7 responded to stimuli on either side of the body or either half of the visual field or they discharged during the monkey s own movements of either side of the body Laterality of the effecti e visual stimuli was tested by diverting the monkey gaze to an other stimulus or by observing the direction of gaze dur ing repetitive stimulations The other half of the cell were activated only by stimuli on the contra-



6 Responses of cell to different visual stimuli Signal from vertical eye movements under the cellular activity ...lateral side of the monkey C the erect tube moving in the midsagittal plane towards the monkey D and E ...tion of the tube in horizontal plane at eye level on the ipsilateral side of the monkey F monkey manipulates ...of leather with the eyes open G manipulation with the eyes covered

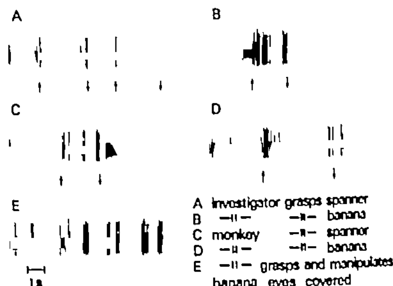


Fig 7 Discharges of a cell in different conditions. Arrows in the figure indicate taking hold of an object with hand (↑) and relea ing of the object (↓)

lateral side of the body (in reference to the hemi sphere being recorded) or visual field or during movements of the contralateral side of the body (Table 2) No cells were activated only by ipsilateral stimuli When a cell responded to somesthetic stimuli on both sides it usually responded more strongly to contralateral stimuli

Referent body part

The functions of most cells which responded to somesthetic stimuli or were active only during the monkey s own movements were related to the sensory motor control of the arms and hands i e they had a receptive field on the arm or hand or were active during grasping manipulation or picking the fur with the fingers (Table 3) Twelve cells had their receptive fields on the head 5 on the shoulder and 7 on the legs No receptive fields were found on the trunk

Table 2 *Laterality of the receptive fields and body parts whose movements were related to the cellular activity in the posterolateral part of area 7*

	No of cells	%
Contralateral	56	50
Ipsilateral	–	
Bilateral	57	50
Total	113	100

DISCUSSION

Results compared with those obtained from neighbouring areas

The area examined is cytoarchitecturally pa[illegible] area 7 of Brodmann (1905) and Vogt & Vogt [illegible] and areas PF and PG of von Bonin & Bailey [illegible] It borders medially and anteriorly on the rest of [illegible] 7 and laterally on the parietal retroinsular [illegible] within the Sylvian fissure and on the parie[illegible] poral association area lateral to the Sylvian f[illegible] Functionally the area examined resembles the [illegible] terolateral part of area 7 which has been mo[illegible] investigated (Leinonen et al 1979 Leinonen [illegible] Nyman 1979) in the following respects (1) the[illegible] respond both to somatosensory and visual st[illegible]

Table 3 *Location of the somesthetic recep[illegible] fields and the body parts whose movements [illegible] related with the cellular activity in the p[illegible] lateral part of area 7*

	No of cells	%
Arm and hand	55	68
Head	1[illegible]	[illegible]
Shoulde[r]	5	[illegible]
Legs	7	[illegible]
Shoulder and hip	1	1
Hand and feet	1	[illegible]
Total	81	100

me cells discharge only during the monkey movements (3) many cells have bilateral re e fields or are active during movement of r side of the body The functions of most cells anterolateral part of area 7 were related to control of face and arm movement e g reaching the lips or arm, bringing an object to the h. hereas functions in the area described in work were mainly related to the control of movements i e grasping and manipulation cells in the neighbouring parietal retroinsular x (area relpt, Pandya & Sanides 1973) respond esthetic stimulation, most of them only to ression of the skin in n on, off or on–off m (Leinonen 1979) In the present study some responded in a similar fashion to similar stimu- The functional resemblance may be due to ntation from area relpt to the area examined tional properties of the cells in the neighbour temporoparietal association cortex (area Tpt ya & Sanides 1973) include responsiveness esthetic stimuli on the temple shoulder or responsiveness to auditory stimuli some cell arge only during the monkey s own head ments (Leinonen et al. in preparation). ls from area Tpt suggest that it participates the control of head movements. In the present some cells in the transitional region between 7 and Tpt were activated by touching of the le and visual stimuli approaching the temple h in natural condition would trigger turning of head towards the stimulus. Thu it can be med that these cells are part of the system rolling the head movements.

active only during the monkey movement

s active only during the monkey s own move s have been reported in several works con- ed in area 7 (Hyvarinen & Poranen 1974 Mountcastle et al. 1975 Lynch et al 1977 Leinonen et al. 1979 Leinonen & Nyman 1979) It has ever not yet been settled whether these cells respond to stimuli which cannot be produced ively (2) are functionally dependent on the ity of the motor and premotor cortex (corollary harge gating phenomenon) (3) participate in analysis of the information content of a stimulus ation and in setting a goal for an action, (4) neurones of the motor system. Area 7 has sev- l connections with areas w re some or all cells discharge only during active movement (1) motor areas arm region in the primary motor cortex send afferents to the lateral part of are 7 (Pandya & Kuypers 1969) ( ) frontal association area area 7 project to the prefrontal cortex (Chavi & Pandya 1976) and receives afferents from there (Stanton et al 1977), (3) the ventrolateral nucleus of the thalamus efferents from area 7 have been described by Kaas et al (1977) afferent to area 7 by Baleydier & Mauguiere (1977) and Stanton et al (1977) The several anatomic connections with the motor control system ndicate that the function in the parietal association cortex depend on motor activity On the other hand some pyramidal tract fibers originate from neurones of area 7 (Peele 194) which suggests that the functions of area 7 are necessary for some mo ements. Examination of these problem was not possible with the methods of the present study

Intersensory sociation

Many cells responded to touching of the hand (when the monkey eyes were covered) and to isual stimuli approaching the arm or hand These cells were usually active also when the monkey himself w reaching for or manipulating an object Apparently these cellular functions were related to the analysis of the direction, location and distance of a moving visual stimulus in reference to the body Thus it can be suggested that the area examined participates in the spatial control of hand movements in the somesthetic and visual paces.

It has been shown that monkeys succeed in cross-modal recognition (visuo-tactile) of objects (Cowey & Weiskrantz 1973 Jarvis & Ettlinger 1977 Bolster 1978) Some findings of the present study suggest that area 7 may participate also in cross-modal recognition of manipulable objects. some cells were active when the monkey manipulated an object with its eyes covered and also when an object was rotating in the visual field.

Result in the light of ablation tudie and electric stimulation xperiments

Ablation of the parietal association cortex of the monkey result in deterioration of several different functions Some of these functions were observed to be related to cellular activity of the part of area 7 examined. somesthetically guided finger movements and tactual recognition of food (Peele 1944) opposition of the thumb and forefinger during grasp-

ing (Faugier-Grimaud et al 1978) tactile shape discrimination (Ruch et al 1938 Bates & Ettlinger 1960 Ettlinger & Kalsbeck 1962) It could be suggested that a lesion in the area examined would result in deterioration of these functions

Electric stimulation of different parts of the parietal association cortex causes various movements of the eyes and arms stimulation of the cortex above the end of the Sylvian fissure produces movements of the hand (Vogt & Vogt 1919 Lilly 1958) which is in accordance with the results of the present study

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spiratory effects elicited in newborn animals the central chemoreceptors

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central chemoreceptors on the ventral surface e medulla oblongata play an important role in ratory control in the adult Increased CO_2 ten- in the blood leads to an increase in the cerebro- l fluid (CSF) H concentration, exciting the al chemoreceptors leading to an increased res- ory drive and vice versa (for review see e.g. chke 1979). The present experiments aimed udying the effects on respiration that can be ed via the central chemoreceptors in the new animal and thus get a hint as to their impor e for the establishment and maintenance of a al and regular respiration in the newborn. dic breathing is not seldom seen in newborn ls, especially those born pre term (e g. tern 1978) and is sometimes a forerunner to ic spells. Increased O_2 tension as well as in- ed CO_2 tension counteracts periodic breath- Recently Cherniack et al. (1979) ha e dem- rated in the adult cat that local cool ng of the ensitive areas on the ventral medulla surface ere the afferent fibres from the adjacent rostral caudal chemoreceptor areas have been sug- ed to converge potentiates periodic breath- One aim of the present experiments was to stigate whether periodic breathing can be pro- ed in the new born animal by inhibiting the cen- chemoreceptors.

experiments have been performed on new born rab- relatively immature at birth, and on new born ea-pigs, mature at birth. The rabbits were anes- ized ith urethan (usually 1 000 mg/kg b wt. p addi- al doses given hen needed) the guinea-pigs with bination of chloralose (25 mg/kg b wt. i p) and urethan mg/kg b wt. i p). The animals were tracheotomized spontaneously breathing The trachea, esophagus and cervical muscles were divided and the frontal aspect the atlas vertebra nibbled away. The atlanto-occipital ane was carefully separated from the dura and made through the dura and arachnoid A fine catheter 1 10 was inserted through this hole with the tip placed just over the chemosensitive zones. Via this catheter the chemoreceptor zones could be superfused with mock CSF of different pH or with GABA (0.1 or 1 mg/ml) dissol ed in mock CSF In some experiment near term guinea-pig fetuses were exteriorized and the above preparation per formed with the umbilical cord circulation still intact and before the breathing had been established Mock cerebro- spinal fluid were prepared according to Mitchell et al (1963) and bubbled with CO_2 to decided pH alues. Respi- ration was recorded a thread sew to the abdominal skin just below the sternum and connected to a force-dis- placement transducer writing on a Gra polygraph. In some of the experiment arterial blood pressure and heart rate were monitored a a catheter cannulating the left carotid artery Periodic breathing was defined as res- piratory pause of 3 or more followed by breathing period of 20 s or les ith the cycle repeated at least three times during min

The central chemoreceptors were found well-func tioning from the first day of life both in the rab- bits and the guinea-pigs. Pronounced respiratory changes were elicited ranging from strong augmen- tation of the ventilation when superfusing the cen- tral chemoreceptors with acid CSF to inhibition of the respiration and even apnea with alkaline CSF (Fig. 1) Decreases in heart rate and blood pressure accompanied the inhibitory effects on respiration. Application of GABA elicited prompt inhibition of tidal volume and respiratory rate accompanied by decreased heart rate and blood pressure fall (cf Wennergren & Öberg 1980) and easily induced apnea. In the exteriorized guinea-pig fetuses clear cut respiratory effects could be induced via the central chemoreceptors immediately after the cord had been clamped and air breathing movements initiated (cf Herrington et al 1971)

Fig 2 demonstrates how inhibition of the central chemoreceptors leads to periodic breathing. When the inhibition is relieved the respiration becomes regular again (top panel) When the sequence is repeated with increased F_IO_2 the inhibition of the

ing (Faugier-Grimaud et al 1978) tactile shape dis crimination (Ruch et al 1938 Bates & Ettlinger 1960 Ettlinger & Kalsbeck 1962) It could be sug gested that a lesion in the area examined would result in deterioration of these functions

Electric stimulation of different parts of the parietal association cortex causes various move ments of the eyes and arms stimulation of the cor tex above the end of the Sylvian fissure produces movements of the hand (Vogt & Vogt 1919 Lilly 1958) which is in accordance with the results of the present study

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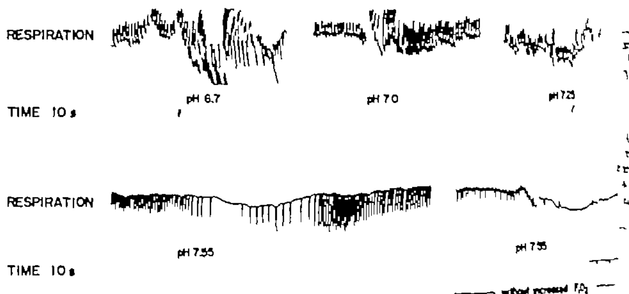


Fig 1 Recordings of respiration in a newborn rabbit of a few h age Note pronounced excitation of the respiration by superfusing the central chemoreceptors with acid CSF and inhibition with alkaline CSF In the last sequence the animal breathes room air In the preceding ones 40% oxygen

central chemoreceptors only leads to reduction of the ventilation but the regular pattern is maintained (middle panel) The same response patterns were seen in newborn guinea pigs Raising F_IO_2 during

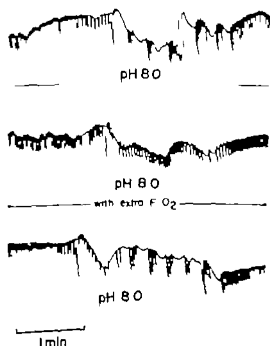


Fig 2 Recordings of respiration in a rabbit age 1 week Inhibition of the central chemoreceptors leads to the development of periodic respiration (top and bottom panel) When the inhibition is performed with the animal breathing 40% oxygen instead of room air a regular breathing pattern is maintained (middle panel).

periodic breathing reversed the pattern to reg... Malfunctioning central chemoreceptors and a ...cient respiratory drive from them may be a mechanism leading to periodic breathing also in the newborn infant Interestingly a significantly augmented frequency and duration of periodic breathing during sleep recently was reported in infants who had had near-miss sudden death episodes (Kelly & Shannon 1979) The authors discussed malfunction at the central chemoreceptor level as one possible explanation of their finding.

In conclusion the present experiments demonstrate well-functioning central chemoreceptors from the first day of life in both rabbits and guinea pigs Further study is needed to elucidate what part the central chemoreceptors play in the establishment of a regular respiration at birth. Experiments on these lines are in progress It is possible to produce periodic breathing in these newborn animals by inhibiting the central chemoreceptors suggesting that impaired respiratory drive from the central chemoreceptors may be one cause of periodic breathing also in the newborn infant. An animal model is here provided for the production and ... of periodic breathing in the newborn

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[illegible]it of tryptophan and phenylalanine from bovine [illegible]rebral cortex synaptosomes

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[illegible] mechanisms of aromatic amino acids have [illegible]to been studied in detail only with brain slices [illegible]bon et al. 1970 Crnic et al. 1973 Laakso 1978 [illegible] & Oja 1979a b) An interpretation of data [illegible] slice work is complicated by the morpho[illegible]cal complexity and cellular heterogeneity of [illegible]es A specific transport mechanism has been [illegible]posed for L-tryptophan in synaptosomes (Deni[illegible] & Sourkes 1977 Mandell & Knapp 1977), [illegible] has never been characterized in relation to [illegible]transport of for instance other aromatic amino [illegible]. A characterization of the efflux and exchange [illegible] tryptophan and phenylalanine has now been [illegible] in isolated superfused bovine brain synapto[illegible].

[illegible]ynaptosomes were isolated and purified from [illegible] bovine cerebral cortices by ultracentrifugation [illegible] sucrose gradients as described by Kontro & Oja [illegible]. All subsequent experiments were carried on the same day as the isolation of synapto[illegible]. The synaptosome fractions were metaboli[illegible] viable and consumed oxygen in Warburg [illegible] for at least 2 h at a constant rate of 0 48±0.05 [illegible] ± S E [illegible] =5) mmol/s × kg protein. Synapto[illegible] samples for electron microscopy were pre[illegible]ed as described (Kontro & Oja 1978). The [illegible]ptosome fractions were fairly homogeneous [illegible]sisting mainly of torn-off nerve ending particles [illegible] which synaptic [illegible]esicles and mitochondria could [illegible] easily identified (Fig. 1).

[illegible]ynaptosome suspensions (2 g protein/l) were [illegible]loaded for 15 min in Krebs-Ringer HEPES-glu[illegible] medium buffered to pH 7 4 at 310 K under O_2 [illegible] 5 [illegible] μCi/l (0 05 mmol/l) L-[G-^{3}H]tryptophan or [2,3-^{3}H]phenylalanine Synaptosomal particles [illegible] 0 5 ml portions of the suspension were layered [illegible] Millipore filters (pore size 0.8 μm, Ø 25 mm) by [illegible]ration Another moistened filter was gently [illegible]ced over the synaptosome layers and such as[illegible]mblies were thereafter transferred into superfu[illegible] chambers built from Swinnex-25 (Millipore) filter holders by reducing their internal volumes with plastered dental acrylic to 0 6 ml Synapto[illegible]somes were first rinsed with 5 ml superfusion solu[illegible]tion to remove extra synaptosomal label and super[illegible]fusion experiment were thereafter carried out as described (Korpi & Oja 1979a) Semilogarithmic efflux curves constructed for [^{3}H]tryptophan and [^{3}H]phenylalanine were subjected to a graphical compartmental analysis (Solomon 1960) as depicted in Fig [illegible] Two components were extracted from the total efflux

Tryptophan was more effectively retained by synaptosomes than phenylalanine during 30-min superfusions with amino-acid-free media (Fig [illegible]). The half-times of the two efflux components were approximately the same for both amino acids (5 and [illegible]0 min) but the share of the slow component was three times larger in tryptophan than in phenylala[illegible]nine efflux These two components could originate from different synaptosomal compartments, but a more plausible explanation is that they represent separate efflux processes. The phenylalanine mole[illegible]cule is less hydrophobic than the tryptophan mole[illegible]cule (Bigelow & Channon 1976) and this circum[illegible]stance may underlie the faster loss of phenylalanine from synaptosomes Phenylalanine release is faster than tryptophan release also in rat cerebral cortex slices (Laakso 1978)

The effects of exogenous unlabelled tryptophan phenylalanine and histidine on [^{3}H]tryptophan efflux are depicted in Fig. 3 Tryptophan efflux was stimulated significantly more from the extracellular side by these amino acids than phenylalanine efflux (Table 1) This indicates that intrasynaptosomal tryptophan is more accessible for exchange with extracellular amino acids than phenylalanine Spon[illegible]taneous efflux of phenylalanine may also be non[illegible]mediated to a greater extent than tryptophan efflux, as is also suggested by the larger share of the fast component in phenylalanine efflux (Fig. 2) and by the greater rate constants for spontaneous efflux

Table 1 *Effects of tryptophan phenylalanine and histidine on the efflux and exchange of [³H]tryptophan and [³H]phenylalanine in synaptosomes from bovine cerebral cortex*

The results (means ± S E) give the efflux rate constants for the superfusion intervals indicated from experiments similar to those depicted in Fig 3 Student s *t* test was used to assess significance of differences from the controls
$P<0.05$ $P<0.01$

Amino acid present (1 mmol/l) from 18 to 22 min	No of expts	Efflux rate constants (×10 min ¹) for times stated		
		1 –16 min	18–22 min	26–30 min
Efflux of [H]tryptophan				
None (control)	3	17 1±0 9	16 0±0 9	1.3±0 6
Tryptophan	4	15.5±1 7	73 7±5.5	11.±1.2
Phenylalanine	4	17 8±1 3	66.0±5.4	11.8±2 1
Histidine	4	15 4±1 8	67.3±8.3	9.5±0 9*
Efflux of [H]phenylalanine				
None (control)	3	27.3±1.2	23 2±1.5	11.8±1 4
Tryptophan	4	26 1±2.0	48.8±7.2	20.5±1.2**
Phenylalanine	4	27 4±2 6	48 2±2 4	4.2±4.2*
Histidine	4	26 0±1 2	43.6±1 8*	15 9±2.1

(Table 1) The elevated efflux of phenylalanine still persisted when the medium was again amino-acid free while the efflux of tryptophan soon returned to the control levels It is thus possible that phenylalanine efflux was also enhanced by intrasynaptosomal amino acids whereas tryptophan efflux was not influenced or even decreased in the case of histidine In general no systematic significant diffe

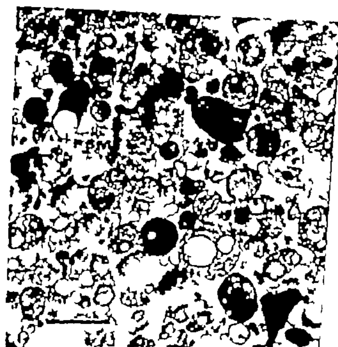


Fig 1 Electron microscopy of bovine cerebral cortex synaptosomes Synaptosomes surrounded by presynaptic membranes and containing synaptic vesicles mitochondria and some vacuoles are visible Electron opaque (arrows) and dense-cored (arrowheads) vesicles can be distinguished There are some unidentified membrane profiles as contaminants. *S* synaptosome *PSM* post synaptic membrane *M* mitochondrion Calibration bar 1 µm.

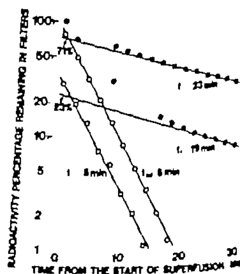


Fig 2 Resolution of the efflux curves of [³H]tryptophan and [³H]phenylalanine into 2 components in synaptosomes from the bovine cerebral cortex Synaptosomes were superfused with plain Krebs-Ringer HEPES phosphate medium for 30 min The percentage of [³H]tryptophan (■) or [³H]phenylalanine (●) remaining in synaptosome layers on Millipore filters is shown as a function of superfusion time in a semilogarithmic plot Each point is a mean of 5 expts Relative S E less than 10% Open symbols define the fast efflux component of [H]tryptophan (□) and [H] phenylalanine (○) efflux remaining after extraction of the slow component from the total efflux The percentage figures in the graph denote the shares of the efflux components The *t*½ values give the half times of the efflux components



J Effects of exogenous unlabelled amino acids on efflux of [³H]tryptophan from bovine cerebral cortex synaptosomes. Synaptosomes were preloaded and superfused as described except that at 17.5 min from the beginning the amino-acid free superfusion solution was exchanged for 4 min with the same solution supplemented with 1 mmol/l unlabelled tryptophan (□), phenylalanine or histidine (○) or with the same unsupplemented solution (●). During this period there occurred exchange of intrasynaptosomal [³H]tryptophan with unlabelled extrasynaptosomal amino acids. At the end of superfusion [³H]tryptophan was released again into amino-acid-free medium together with the newly accumulated unlabelled amino acids. The graph gives representative examples from 4 parallel expts. The straight lines are computed regression lines for the data of the 3 successive experimental phases (Korpi & Oja 1979b). The negative slopes of the lines are the efflux rate constants compiled in Table 1.

...as could be demonstrated in the effects of tryptophan, phenylalanine and histidine on the efflux processes, although all efflux rate constants during and after superfusions with histidine tended to be numerically slightly smaller.

Trans-stimulation of efflux by extrasynaptosomal amino acids nicely demonstrates the participation of membrane transport sites in the stimulated efflux of tryptophan and phenylalanine. No fundamental differences in the efflux mechanisms of tryptophan and phenylalanine were revealed in the present study. This circumstance does not corroborate the assumption of a transport mechanism specific for tryptophan in nerve endings. Our results from bovine cerebral cortex synaptosomes are apparently not at variance with the results of earlier studies on rat brain slices (Laakso 1978, Korpi & Oja 1979a, b).

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Table 1 *Effects of tryptophan phenylalanine and histidine on the efflux and exchange of [³H]tryptophan and [³H]phenylalanine in synaptosomes from bovine cerebral cortex*

The results (means ± S E) give the efflux rate constants for the superfusion intervals indicated from experiments similar to those depicted in Fig 3 Student s *t*-test was used to assess significance of differences from the controls. $P<0.05$ $P<0.01$

Amino acid present (1 mmol/l) from 18 to 22 min	No of expts	Efflux rate constants (×10⁻ min⁻¹) for times stated 12–16 min	18–22 min	26–30 min
Efflux of [³H]tryptophan				
None (control)	3	17 1±0 9	16 0±0 9	12.3±0.6
Tryptophan	4	15 5±1 7	73 7±5.5**	11.2±1.2
Phenylalanine	4	17 8±1 3	66 0±5 4	11.8±2 1
Histidine	4	15 4±1 8	67.3±8.3**	9.5±0.9*
Efflux of [³H]phenylalanine				
None (control)	3	27 3±1.[illegible]	23 [illegible]±1.5	11.8±1 4
Tryptophan	4	26 1±2 0	48.8±2.2	20.5±1.2*
Phenylalanine	4	[illegible]7 4± [illegible]6	48 2±2.4**	24.2±4.2*
Histidine	4	26 0±1.2	43 6±1.8	15 9±[illegible]1

(Table 1) The elevated efflux of phenylalanine still persisted when the medium was again amino-acid free while the efflux of tryptophan soon returned to the control levels It is thus possible that phenylalanine efflux was also enhanced by intrasynaptosomal amino acids whereas tryptophan efflux was [illegible] influenced or even decreased in the case of histidine In general no systematic significant diff[illegible]



Fig 1 Electron microscopy of bovine cerebral cortex synaptosomes Synaptosomes surrounded by presynaptic membranes and containing synaptic vesicles mitochondria and some vacuoles are visible Electron opaque (arrows) and dense-cored (arrowheads) vesicles can be distinguished. There are some unidentified membrane profiles as contaminants. *S* synaptosome *PSM* postsynaptic membrane *M* mitochondrion. Calibration bar 1 µm

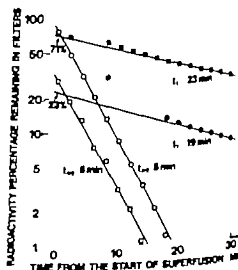


Fig 2 Resolution of the efflux curves of [³H]tryptophan and [³H]phenylalanine into 2 components in synaptosomes from the bovine cerebral cortex Synaptosomes were superfused with plain Krebs-Ringer HEPES glucose medium for 30 min The percentage of [³H]tryptophan (■) or [³H]phenylalanine (●) remaining in synaptosome layers on Millipore filters is shown as a function of superfusion time in a semilogarithmic plot Each point is a mean of 5 expts Relative S E. less than 10% Open symbols define the fast efflux component of [³H]tryptophan (□) and [H] phenylalanine (O) efflux remaining after extraction of the slow component from the total efflux. The percentage figures in the graph denote the shares of the slow efflux components The *t* ½ values give the half-times of efflux components.

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…oduction and evaluation of antibodies to glucagon albumin …njugates in the guinea pig

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…ough various techniques have been reported for …production of glucagon antisera, the stimulation …ntibodies …th suitable affinity and specificity …use in the radioimmunoa…say of glucagon ha… …particularly difficult to achie…e (Heding 1977) …nsistent with the unique structural and im…nological properties of guinea pig glucagon (As…Tchobroutsky & Rosselin 1969 Sundby 1976) …ious studies have underlined the particular …al of the guinea pig for the rapid production of …ent glucagon antisera against glucagon-carbod…de-albumin conjugates (Flatt & Swanston-…tt 1979) Since there is evidence that the use …glutaraldehyde as the coupling agent result… the production of antisera with restricted …ecificity in the rabbit (Garaud et al. 1976) groups …guinea pigs were immunized with glucagon-…umin conjugates prepared at an approximate mo…ratio of 10 1 using either carbodiimide or glutar…ldehyde as the coupling agent.

Carbodiimid… conjugation 50 mg crystalline …ine glucagon (Calbiochem) was dissolved in 0.3 … hydrochloric acid. To this was added 200 pg …I-glucagon and 62 5 mg bovine plasma albumin in … 5 ml water The solution was made up to 16 ml …d 300 mg 1-ethyl-3-(3-dimethyl-aminopropyl)-car…bodiimide hydrochloride (Sigma) was added drop…wise in 1 ml water The mixture was stirred at room …temperature for 24 h after which the preparation …was extensively dialysed. The extent of coupling …was approximately 80 %

Glutaraldehyde conjugation 10 mg crystalline …porcine glucagon (Novo) 200 pg ^{125}I-glucagon and …2.5 mg bovine plasma albumin were mixed in 4 ml …sodium phosphate buffer (0.2 M pH 7.3) T… this …suspension was added 4 ml 5 % glutaraldehyde The …mixture was stirred at room temperature for 24 h …and then extensively dialysed The extent of cou…pling was approximately 75 %

Immuni…ation Groups of f…e male albino guinea pigs (150 g) were inoculated subcutaneously with either 500 μg glucagon (carbodiimide) or 300 μg glucagon (glutaraldehyde) emulsified in an equal volume of Freund's complete adjuvant Secondary and subsequent injections were administered at adjacent …ites every 4 week… using the same doses of immunogen and incomplete adju…ant The animals were bled by cardiac puncture I… 14 days after the second booster and all subsequent injections

Anti body a…e sment The sera were assessed in terms of titre affinity and …pecificity using a 450 μl equilibrium assay system dependent on 10 pg mono-iodinated porcine glucagon (Jorgensen & Larsen 1977) and charcoal separation Standard curves were prepared using a porcine glucagon standard (MRC London) and the …pecificities of the antisera were evaluated using a gut extract prepared from the entire mouse GI-tract (Kenny 1955) This heterogeneous gut GLI preparation was cal brated using a fully cross reacting antiserum (R59) gener…ously donated by Professor K D Buchanan.

Result… As illustrated in Fig. 1 all guinea pigs immunized with the conjugate prepared using carbodiimide developed large amounts of high af…finity glucagon antibodies which exhibited slightly different degrees of cross reaction with gut GLI Antibody titre and affinity rose progressively during immunization attaining very high values by 22 weeks In contrast the conjugate prepared using glutaraldehyde was markedly less effective in stimulating antibody production in the guinea pig. Thus of the 5 animals inoculated over a period of approximately 6 months, only one responded by producing glucagon antibodies (Fig. 1). The sera

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TITRE AFFINITY SPECIFICITY

8 75 3

8

4 50 2

2 25 1

0 0 0

Fig 1 Characterization of glucagon antisera from guinea pigs immunized with glucagon-albumin conjugates prepared using carbodiimide (open circles) or glutaraldehyde (closed circles) Values for each animal are given as mean of 4 bleedings taken over a period of 22 weeks The mean result for each group is indicated by the horizontal bar Titre log-inverse 50% maximum binding dilution Affinity- % fall bound counts caused by 125 pg glucagon 100 (Bo-B)/Bo Specificity- ratio of tracer displacement caused by 125 pg glucagon and 125 pg equiv gut GLI

derived from this particular animal contained antibodies of relatively high affinity which displayed partial discrimination between glucagon and gut GLI (Fig 2) Antibody titres were slightly below average in this guinea pig and like affinity rose only gradually during immunization

Discussion Monthly immunization of guinea pigs with a glucagon-carbodiimide albumin conjugate was associated with the successful production of large amounts of high affinity glucagon antibodies Indeed in a comparative study it has previously been suggested that the enhanced immunogenicity of such conjugates in the guinea pig makes this animal more attractive than the rabbit for the rapid production of potent glucagon antisera (Flatt & Swanston-Flatt 1979) However antisera raised in the guinea pig against the conjugate prepared using carbodiimide appear to invariably react almost equally well with glucagon and gut GLI Thus although such potent antisera are particularly valuable for many studies they cannot justifiably be used for the assay of glucagon in plasma or other samples which contain additional GLI components

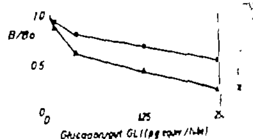


Fig 2 Reaction of the sole responder in the group of guinea pigs immunized with the glucagon-glutaraldehyde albumin conjugate (GPD4/3) with glucagon (triangles) and gut GLI (squares)

The C-terminal portion of the glucagon molecul is believed to represent the specific immunogenic determinant of antibodies which react poorly wi gut GLI (Assan & Slusher 1972 Ohneda et a 1979) Accordingly glutaraldehyde which probabl conjugates albumin to the amino groups at residu 1 and 12 of the N terminal fragment of glucagon h been reported to exclusively stimulate the produc tion of antisera with restricted specificity in th rabbit (Garaud et al 1976) Nevertheless it was n possible to achieve a similar degree of success wi such a conjugate in the guinea pig. Indeed the u of a glucagon-glutaraldehyde-albumin conjugate i the present study was not associated with the pr duction of particularly valuable glucagon antisera The sole responder yielded high affinity antibodie which in accordance with the experience o Frohman et al (1970) displayed partial discrimina tion between glucagon and gut GLI Thus, althoug the guinea pig represents a particularly usef species for the rapid production of potent hig affinity antisera against glucagon-carbodiimide protein conjugates antibody specificity remains problem in this species

Highly purified crystalline porcine glucagon for radio iodination was kindly supplied by Dr Mary A Root of Lilly Research Laboratories Indianapolis Indiana, USA

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apid simultaneous determination of regional blood flow ıd blood-brain glucose transfer in brain of rat[1]

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GJEDDE, A., HANSEN A J & SIEMKOWICZ E. Rapid simultaneous determination of regional blood flow and blood brain glucose transfer in brain of rat. Acta Physiol Scand 1980, 108 321 330 Received 17 Jan 1979 ISSN 0001-6772 Institute of Medical Physiology Department A, University of Copenhagen Denmark.

A new method was dev loped and used in rat to measure regional and whole-brain blood flow and blood-brain glucose transfer simultaneously and in 20 This simple method consisted of bolus injection of labeled butanol and tracer glucose determination of the average arterial tracer concentration and subsequent assay of cerebral tissue act ity 20 after bolus injection The whole-brain blood flow rate eraged 179 ml (100 g) min The unidirectional blood brain glucose transfer was twice as high as previously estimated in similar studies on rat, or 144 μmol (100 g) min at 10 mM glucose in plasma The magnitude is sufficient to explain the high cerebral glucose consumption rates recently deter mined by means of the autoradiographic 2-deoxy D-glucose method of Sokoloff et al (1977)

Key ord Regional cerebral blood flow blood-brain glucose transfer integral method

nt methods for determination of regional cere l blood flow in the rat require determination of erial, tissue, and/or cerebral venous indicator ncentrations over a period of at least 60 and st methods for determination of whole-brain ad flow require equilibration for at least 10 min, hough shortening of the time to 3 min has been cessfully achieved under conditions of very high ood flow rates

In non-steady-states, procedures of shorter dura- n may be necessary Such an approach is de ribed below The method is an extension of the tegral method of Landau et al. (1955) and permits hole brain and regional cerebral blood flow rates be measured by a procedure lasting less than 10 s. In addition, we extended the method to involve e simultaneous but independent determination of nidirectional blood-brain glucose transfer rates Glucose is the major nutrient of the brain and is nsported from blood to brain tissue by facilitated ffusion. The unidirectional flux has never previ- usly been measured rapidly under approximate- y steady-state conditions, and the response of blood-brain glucose transport to acute alterations of brain function is therefore unknown.

In brief the method is applied in the following ay A bolus injection of labeled butanol and tracer glucose is given in a femoral vein. Arterial blood is slowly withdrawn into syringe for 10 or 20 from a femoral artery At the end of the 10 or 20 s sampling is discontinued the rat is decapitated and the brain removed divided and deposited in closed vials for further processing.

THEORY

Determination of cerebral blood flow

The fundamental equation used to measure cerebral blood flow by means of indicator fractionation is the following.

$$F^{bl} = \frac{C_{br}(T)}{E(T)\int_0^T C^{bl}(t)\,dt} \quad (1)$$

in which F^{bl} is the blood flow rate per unit weight of sample of brain, $C_{br}(T)$ the indicator content per unit weight of brain tissue $E(T)$ the net extraction fraction of indicator in the time from introduction of the indicator into the circulation (t 0) to the termination of the expt (t=T) and $C^{bl}_{a}(t)$ the arterial blood concentration of the indicator at the time t. The usefulness of equation (1) depends entirely on the knowledge of E(T). The assumptions underlying the equation its development and methods of estimation of E(T) will be presented below

[1] Presented in part at the 28th Annual Meeting of the American Academy of Neurology in Toronto Canada, April 1976 (Schaefer et al. 1976).

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Table 1. *Net cerebral extraction fraction of freely diffusible indicator in brain as function of arterial concentration*

	Function	E(T)
	$C^{M}(t)$	$\dfrac{\exp\left[\dfrac{f^{M}T}{\lambda_{br}}\right]\int C^{M}(t)\exp\left[\dfrac{f^{M}t}{\lambda_{br}}\right]dt}{\int^{T} C^{M}(t)\,dt}$
	k	$\dfrac{1-\exp\left[-\dfrac{f^{M}T}{\lambda_{br}}\right]}{\left[\dfrac{f^{M}T}{\lambda_{br}}\right]}$
	kt	$\dfrac{}{\left[\dfrac{f^{M}T}{\lambda_{br}}\right]}\left[1-\dfrac{1-\exp\left[-\dfrac{f^{M}T}{\lambda_{br}}\right]}{\left[\dfrac{f^{M}T}{\lambda_{br}}\right]}\right]$
exponential	$C^{M}(t')\exp[-k(t-t')]$, $t\geq t'$	$\dfrac{k}{\left[\dfrac{f^{M}}{\lambda_{br}}-k\right]}\left[\dfrac{\exp[-k(T-t')]-\exp\left[-\dfrac{f^{M}(T-t')}{\lambda_{br}}\right]}{1-\exp[-k(T-t')]}\right]$

its entirety (i.e. to time T) monoexponential decay from time t', i.e. $C_a^{M}(t) = C_a^{M}(t')\exp[-k(t-t')]$ ... then, as shown in Table 1 for $k \neq p$

$$E_f = \frac{k\{\exp[-k(T-t')]-\exp[-p(T-t')]\}}{(p-k)(1-\exp[-k(T-t')])} \qquad (9)$$

which p equals f^{M}/λ_{br}. However the $C^{M}(t)$ curve rises ... peak time t'. For E(T) calculated from equation (9) ... be an acceptable approximation of the actual E(T) for ... period 0 to T, it must therefore be shown that the ... of labeled butanol in arterial blood prior to t' ... not cause E(T) calculated by means of equation (9) to ... greatly in error. It will be shown in Results that this ... is the case for T 20. By insertion of equation (9) ... equation (7) it is possible to express the brain content of ... as a function of p, k, and T

$$\frac{k\{\exp[-k(T-t')]-\exp[-p(T-t')]\}}{(p-k)(1-\exp[-k(T-t')])} = \frac{F^{M}C_{br}(T)}{\lambda_{br}Q_a(T)} \qquad (10)$$

... which equation f^{M} can be calculated when F^{M}, $C_{br}(T)$, λ_{br}, $Q_a(T)$, k, T and t' are known, provided the assumptions underlying the equations (4) and (8) are reasonably fulfilled.

In the present study the equations were used as follows. Initially k and t' were estimated from values of $C^{M}(t)$. Second, p was estimated by fitting equation (9) to the experimentally determined values of E(T). Third, f^{M} determined from equations (1) and (5) was compared to f^{M} calculated from equation (10) using a value of λ_{br} obtained directly by equilibration of blood and brain tissue with labeled butanol.

Two problems interfere with the calculation of p (and hence f^{M}) from equation (10): First, the method as presented does not yield k and t', and second, equation (10) is a transcendental equation which can be satisfied by one, two, or no positive values of p. The problems were addressed as follows.

In routine application of the method, the constants k and t' are estimated by the simple expedient of measuring the arterial indicator concentration, $C_a^{M}(T)$, at the time of termination of the expt. If

$$C^{M}(t) = C^{M}(t')\exp[-k(t-t')] \quad t\geq t'$$

then

$$C_a^{M}(T) = C_a^{M}(t')\exp[-k(T-t')]$$

and by insertion of the expression for $C_a^{M}(t)$ derived from these two equations into equation (6), with $C_a(T)$ replaced by $Q_a(T)$, f^{M} by F_a^{M} and $E_a(T)$ by 1

Following introduction of an indicator into the circulation the indicator is taken up per unit weight of any organ including brain at a rate equal to the product of the blood flow rate per unit organ weight f^{bu} and the arteriovenous deficit

$$\frac{dC_{br}(t)}{dt} = f^{bu}\left(C_a^{bu}(t) - \bar{C}_v^{bu}(t)\right) \quad (2)$$

where $\bar{C}^{bu}(t)$ is the organ venous blood concentration of the indicator at the time t after introduction of the indicator into the circulation When brain uptake of the indicator is entirely flow-limited i e equilibrium between tissue and venous concentrations occurs instantaneously the rate of change of brain indicator content can be expressed by the following modification (Kety 1951) of equation (2)

$$\frac{dC_{br}(t)}{dt} = f^{bu}\left(C^{bu}(t) - \frac{C_{br}(t)}{\lambda_{br}}\right) \quad (3)$$

in which λ_{br} is the tissue-blood partition coefficient of the indicator (ml g) Provided f^{bu} is constant and the tissue is homogeneous with respect to f^{bu} and λ_{br}, equation (3) can be integrated to yield

$$C_{br}(T) = f^{bu}\exp\left[-f^{bu}\frac{T}{\lambda_{br}}\right]\int C^{bu}(t)\exp\left[f^{bu}\frac{t}{\lambda_{br}}\right]dt \quad (4)$$

Equation (4) was used by Landau et al (1955) to measure regional cerebral blood flow in the cat 60 s after an i v bolus injection of antipyrine In this case tissue concentrations were measured autoradiographically as was λ_{br}, in regions of brain sufficiently small to allow the authors to argue that the conditions of integration were fulfilled In the present study λ_{br} and f^{bu} will be measured in regions of brain approximately corresponding to major subdivisions of the rat brain (cerebellar hemispheres vermis pons midbrain basal ganglia, parietal cortex and frontal occipital and temporal lobes) These regions consist chiefly of grey matter for which it has been shown that oxidative metabolism and by inference f^{bu} does not vary much within each region (Sokoloff et al 1977)

E(T) the net extraction fraction of indicator in the zero to T time interval is defined by the following equation

$$E(T) = 1 - \frac{\int^{T} \bar{C}_v^{bu}(t)\,dt}{\int C^{bu}(t)\,dt} \quad (5)$$

Insertion of the numerator from equation (5) in equation (2) after integration readily yield the fundamental equation (1). Equation (1) applies to any organ as well as to the whole body provided f^{bu} is constant Thus for a unit weight of another organ indicated by subscript o

$$c_o(T) = f^{bu} E_o(T)\int_0^T C^{bu}(t)\,dt \quad (6)$$

If $C^{bu}(t)$ for the two organs are identical for all valu of t from 0 to T

$$f^{bu} = f^{bu}\,\frac{E_o(T)}{E(T)}\,\frac{C_{br}(T)}{C_o(T)}$$

In the present method, a reference organ is intr duced in the form of a syringe into which blood is drawn at the rate F^{bu} In this case $C_o(T)$ is substituted by $Q_o(T)$, the total radioactivity accumulated in the syringe in the time 0 to T and $E_o(T)$ equals 1 Thus for the relation between an organ and the syringe we have

$$f^{bu} = F^{bu}\,\frac{C_{br}(T)}{E(T)\,Q_o(T)}$$

Equation (7) is the operational form of the fundam equation of the method In whole-brain studies, E(T) be determined directly by equation (5) following anal of arterial and mixed venous blood. For regional measu ments in which C^{bu} for each region is unknown, E(T) be determined by the following combination of equatio (1) and (4)

$$E(T) = \frac{\exp\left[-f^{bu}\frac{T}{\lambda_{br}}\right]\int C_a^{bu}(t)\exp\left[f^{bu}\frac{t}{\lambda_{br}}\right]dt}{\int C^{bu}(t)\,dt}$$

which rests on the assumptions that underlie equation i e homogeneity of f^{bu} and λ_{br} in the region examined, equilibration of the flow indicator between tissue venous blood and constancy of blood flow during experiment

Equation (8) can be solved numerically if suffic numerous values of $C^{bu}(t)$ are known although it ca difficult to determine such values with sufficient accura On the other hand it is possible to perform the tra injection in a manner which produces an arterial conc tration-time curve approximating a simple continu function which after insertion into equation (8) leads manageable expression. Three experimentally useful fu tions, i e $C^{bu}(t) = kt$ (ramp infusion) $C_a^{bu}(t) = k$ (squ wave) and $C^{bu}(t) = C^{bu}(t')\exp[-\lambda(t - t')]$, (i v venous bolus injection) are listed in Table I with corresponding expressions of E(T) obtained accord equation (8) The square wave and ramp infusion si tions give rise to expressions of E(T) with the bl fl of the brain as the only variable

Ramp and square wave infusions were rejected in t present study however because the simultaneous inv tigation of blood-brain glucose transfer required that t concentration of labeled glucose in the cerebral exch the time of termination of the experiment be maxi Therefore i v bolus injection was used, and the appro mation was made (for experimental confirmation see l ter) that the major part of the $C^{bu}(t)$ concn time curve k butanol closely approximated a monoexponential dec function when butanol wa administered as an i v bolus less than a second Thus, if the $C_a^{bu}(t)$ vs. time curve

[Tab]le 1. Net cerebral [ex]traction fraction of freel[y d]iffusible indicator in brai[n] as function of arterial [conce]ntration

[Functio]n	Function	E(T)
	$C^{*}(t)$	$\exp\left[-\frac{f^{*}T}{\lambda_{w}}\right]\int C^{*}_{a}(t)\exp\left[\frac{f^{*}t}{\lambda_{w}}\right]dt \Big/ \int^{T} C^{*}(t)\,dt$
[consta]nt value	k	$\dfrac{1-\exp\left[-\frac{f^{*}T}{\lambda_{w}}\right]}{\left[\frac{f^{*}T}{\lambda_{w}}\right]}$
[ram]p	kt	$\dfrac{1}{\left[\frac{f^{*}T}{\lambda_{w}}\right]}\left[1-\dfrac{1-\exp\left[-\frac{f^{*}T}{\lambda_{w}}\right]}{\left[\frac{f^{*}T}{\lambda_{w}}\right]}\right]$
[Mon]o-[expon]ential [deca]y	$C^{*}(t')\exp[-k(t-t')]$ $t\geq t'$	$\dfrac{k}{\left[\frac{f^{*}}{\lambda_{w}}-k\right]}\left[\dfrac{\exp[-k(T-t')]-\exp\left[-\frac{f^{*}(T-t')}{\lambda_{w}}\right]}{1-\exp[-k(T-t')]}\right]$

[...]ls entirety (i.e. to time T) a monoexponential decay [...]tion from time t' i.e. $C^{*}_{a}(t) = C^{*}_{a}(t')\exp[-k(t-t')]$ [for t≥]t' then, as shown in Table 1 for k≠p,

$$E(T) = \frac{k\{\exp[-k(T-t')]-\exp[-p(T-t')]\}}{(p-k)\{1-\exp[-k(T-t')]\}} \qquad (9)$$

[wh]ich p equals f^{*}/λ_{w}. However the $C^{*}_{a}(t)$ curv[e] [r]ises [...]ter peak time t'. For E(T) calculated from equation (9) [to] be an acceptable approximation of the actual E(T) for [the p]eriod 0 to T it must therefore be shown that the [...]tent of labeled butanol in arterial blood prior to t' [...] in that case E(T) calculated by means of equation (9) to [...]ously in error. It will be show[n] in Results that this [...] is the case for T≥20 s. By insertion of equation (9) [in equ]ation (7) it is possible to express the brain content of [...] as a function of p, k, and T

$$\frac{k\{\exp[-k(T-t')]-\exp[-p(T-t')]\}}{(p-k)\{1-\exp[-k(T-t')]\}} = \frac{F^{*}C_{w}(T)}{\lambda_{w}\,Q_{a}(T)} \qquad (10)$$

[fro]m which equation f^{*} can be calculated when F^{*}, [C_{w}](T), λ_{w}, Q_{a}(T), k, T and t' are known, provided the [ass]umptions underlying the equations (4) and (8) are [reas]onably fulfilled.

[...]In the present study the equations were used as follow. Initially k and t' were estimated from values of $C^{*}_{a}(t)$. Second p was estimated by fitting equation (9) to the experimentally determined values of E(T). Third f^{*} determined from equations (1) and (5) was compared to f^{*} calculated from equation (10), using [a v]alue of λ_{w} obtained directly by equilibration of blood and brain tissue with labeled butanol.

Tw[o] problems interfere with the calculation of p (and hence f^{*}) from equation (10). First the method as pre[s]ented does not yield k and t' and second, equation (10) is [a] transcendental equation which can be satisfied by one, tw[o] or no positi[ve] values of p. The problems were addressed as follows.

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$$C^{*}_{a}(t) = C^{*}(t')\exp[-k(t-t')],\ t\geq t'$$

then

$$C^{*}_{a}(T) = C^{*}_{a}(t')\exp[-k(T-t')],$$

and by insertion of the expression for $C^{*}_{a}(t)$ derived from these two equations into equation (6) with $C_{w}(T)$ replaced by $Q_{a}(T)$, f^{*} by F^{*}_{w} and $E_{w}(T)$ by 1

Following introduction of an indicator into the circulation the indicator is taken up per unit weight of any organ including brain at a rate equal to the product of the blood flow rate per unit organ weight f^{bl} and the arteriovenous deficit

$$\frac{dC_{br}(t)}{dt} = f^{bl}\left(C^{bl}_a(t) - C^{bl}_v(t)\right) \qquad (2)$$

where $C^{bl}_v(t)$ is the organ venous blood concentration of the indicator at the time t after introduction of the indicator into the circulation. When brain uptake of the indicator is entirely flow-limited i e equilibrium between tissue and venous concentrations occurs instantaneously" the rate of change of brain indicator content can be expressed by the following modification (Kety 1951) of equation (2)

$$\frac{dC_{br}(t)}{dt} = f^{bl}\left(C^{bl}_a(t) - \frac{C_{br}(t)}{\lambda_{br}}\right) \qquad (3)$$

in which λ_{br} is the tissue blood partition coefficient of the indicator (ml g) Provided f^{bl} is constant and the tissue is homogeneous with respect to f^{bl} and λ_{br}, equation (3) can be integrated to yield

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E(T) the net extraction fraction of indicator in the zero to T time interval is defined by the following equation

$$E(T) = 1 - \frac{\int C^{bl}_v(t)\,dt}{\int^T C^{bl}_a(t)\,dt} \qquad (5)$$

Insertion of the numerator from equation (5) in equation (2) after integration readily yield the fundamental equation (1). Equation (1) applies to any organ as well as to the whole body provided f^{bl} is constant Thus for a unit weight of another organ indicated by subscript o

$$c_o(T) = f^{bl}E_o(T)\int^T C^{bl}_a(t)\,dt \qquad (6)$$

If $C^{bl}_a(t)$ for the two organs are identical for all values of t from 0 to T

$$f^{bl} = f^{bl}_o \frac{E_o(T)}{E(T)}\frac{C_{br}(T)}{C_o(T)}$$

In the present method a reference organ is introduced in the form of a syringe into which blood is drawn at the rate F^{bl} In this case $C_o(T)$ is substituted by $Q_o(T)$, the total radioactivity accumulated in the syringe in the time 0 to T and $E_o(T)$ equals 1 Thus for the relationship between an organ and the syringe we have

$$f^{bl} = F^{bl}\frac{C_{br}(T)}{E(T)\,Q_o(T)} \qquad (7)$$

Equation (7) is the operational form of the fundamental equation of the method In whole-brain studies, E(T) can be determined directly by equation (5) following analysis of arterial and mixed venous blood. For regional measurements in which C^{bl}_v for each region is unknown, E(T) can be determined by the following combination of equations (1) and (4)

$$E(T) = \frac{\exp\left[-f^{bl}\frac{T}{\lambda_{br}}\right]\int^T C^{bl}_a(t)\exp\left[f^{bl}\frac{t}{\lambda_{br}}\right]dt}{\int C^{bl}_a(t)\,dt} \qquad (8)$$

which rests on the assumptions that underlie equation (4) i e homogeneity of f^{bl} and λ_{br} in the region examined, instantaneous equilibration of the flow indicator between tissue and venous blood and constancy of blood flow during the experiment

Equation (8) can be solved numerically if sufficiently numerous values of $C^{bl}_a(t)$ are known although it can be difficult to determine such values with sufficient accuracy On the other hand it is possible to perform the tracer injection in a manner which produces an arterial concentration-time curve approximating a simple continuous function which after insertion into equation (8) leads to a manageable expression. Three experimentally useful functions i.e $C^{bl}_a(t) = kt$ (ramp infusion) $C^{bl}_a(t) = k$ (square wave) and $C^{bl}_a(t) = C(t)\exp[-k(t-t)]$ (intravenous bolus injection) are listed in Table 1 with the corresponding expressions of E(T) obtained according to equation (8) The square wave and ramp infusion functions give rise to expressions of E(T) with the blood flow of the brain as the only variable

Ramp and square wave infusions were rejected in the present study however because the simultaneous investigation of blood-brain glucose transfer required that the concentration of labeled glucose in the cerebral vessels at the time of termination of the experiment be minimal Therefore i v bolus injection was used and the approximation was made (for experimental confirmation, see later) that the major part of the $C^{bl}_a(t)$ versus time curve for butanol closely approximated a monoexponential decay function when butanol was administered as an i v bolus in less than a second Thus if the $C^{bl}_a(t)$ vs. time curve

ble 1. Net cerebral e traction fraction of freely diffusible indicator in brain as f nction of arterial ncentration

[illegible]	Function	E(T)
[illegible]	$C_a^{*}(t)$	$\exp\left[\frac{f^{*}T}{\lambda_{br}}\right]\int C^{*}(t)\exp\left[\frac{f^{*}t}{\lambda_{br}}\right]dt \Big/ \int^{T} C^{*}(t)\,dt$
[illegible]	k	$\frac{1 \quad \exp\left[\frac{f^{*}T}{\lambda_{br}}\right]}{\left[\frac{f^{*}T}{\lambda_{br}}\right]}$
[illegible]	kt	$\frac{2}{\left[\frac{f^{*}T}{\lambda_{br}}\right]}\left[1 \quad \frac{1-\exp\left[\frac{f^{*}T}{\lambda_{br}}\right]}{\left[\frac{f^{*}T}{\lambda_{br}}\right]}\right]$
[illegible]	$C_a^{*}(t')\exp[\ k(t\ \ t')]$, $t\geq t'$	$\frac{k}{\left[\frac{f^{*}}{\lambda_{br}}\ \ k\right]}\left[\frac{\exp[-k(T-t')] \quad \exp\left[\frac{f^{*}(T-t')}{\lambda_{br}}\right]}{1 \quad \exp[\ k(T-t')]}\right]$

[illegible] to time T) monoexponential decay [illegible] from time t' e, $C_a^{*}(t) = C_a^{*}(t')\exp[-k(t-t')]$ [illegible] then, as show in Table 1 for $k \neq p$,

$$\frac{k\{\exp[-k(T\ \ t')]-\exp[-p(T-t')]\}}{(p-k)\{1-\exp[\ k(T-t')]\}} \tag{9}$$

[illegible] which p equals f^{*}/λ_{br}. However the $C^{*}(t)$ curve rises [illegible] peak time t' For E(T) calculated from equation (9) [illegible] an acceptable approximation of the actual E(T) for [illegible] period 0 to T it must therefore be shown that the [illegible] of labeled butanol in arterial blood prior to t' [illegible] cause E(T) calculated by means of equation (9) to [illegible] greatly in error It will be shown in Results that this [illegible] the case for T 20 s. By insertion of equation (9) [illegible] equation (7) it is possible to express the brain content of [illegible] as a function of p, k and T

$$\frac{k\{\exp[-k(T\ \ t')]-\exp[-p(T\ \ t')]\}}{(p-k)\{1-\exp[\ k(T\ \ t')]\}} \quad \frac{F^{*}C_{br}(T)}{\lambda_{br}Q_a(T)} \tag{10}$$

[illegible] which equation f^{*} can be calculated when F^{*} [illegible](T), λ_{br}, $Q_a(T)$, k, T and t' are known, provided the assumptions underlying the equations (4) and (8) are [illegible] fulfilled.

In the present study the equations were used as follows Initially k and t' were estimated from values of $C_a^{*}(t)$ Second p was estimated by fitting equation (9) to the experimentally determined values of E(T) Third, f^{*} determined from equations (1) and (5) was compared to f^{*} calculated from equation (10), using a value of λ_{br} obtained directly by equilibration of blood and brain tissue with labeled butanol.

Two problems interfere with the calculation of p (and hence f^{*}) from equation (10): First the method as presented does not yield k and t' and second, equation (10) is transcendental equation which can be satisfied by one two or no positive values of p The problems were addressed as follows.

In routine application of the method the constants k and t' are estimated by the simple expedient of measuring the arterial indicator concentration, $C_a^{*}(T)$ at the time of termination of the xpt If

$$C^{*}(t) = C_a^{*}(t')\exp[-k(t\ \ t')],\ t\geq t'$$

then,

$$C_a^{*}(T) = C_a^{*}(t')\exp[-k(T-t')],$$

and by insertion of the expression for $C_a^{*}(t)$ derived from these two equations into equation (6) with $C_a(T)$ replaced by $Q\ (T)$, f^{*} by F_a^{*} and $E_a(T)$ by 1

Table 2. *Comparison of solutions to equations (1) and (5) and equation (10) for T=20 s kt=0.32 s λ_{br}=0 77 ml g⁻¹*

$\frac{F^{bl}C_{br}(T)}{Q_a(T)}$	f^{bl} equations (1) & (5)	f^{bl} equation (10) k=0 04 s	k=0 07 s	k=0 1 s
102±9	133±1	123±13	135±16	147±20

All other values are expressed in ml (100 g) min ±S E. (n=10).

$$\frac{Q\ (T)}{F^{bl}} = \int C_a^{bl}(T)\exp[k(T-t\ )]\exp[-k(t-t\ )]dt$$

or

$$\frac{Q\ (T)}{F^{bl}} = C^{bl}(T)\frac{(\exp[k-Tkt\ ])-1}{k} \quad (11)$$

in which the symbols represent the variables defined above From equation (11) k can be calculated when the variables $Q_a(T)$ F^{bl} and $C^{bl}(T)$ as well as kt are known The value of kt was considered a constant for the following reasons Both k and t are functions of the cardiac output and a central volume of distribution located between the site of injection and the site of arterial blood collection. When the ratio between these two variables increases t becomes smaller and k greater (and the opposite changes occur when the ratio is reduced) The value of kt' probably approximates the ratio between t and the time of passage of blood between the injection and sampling sites

Thus only two constants need to be known in advance of blood flow measurement kt and λ_{br}

The second problem involves the number of solutions to equation (10) When $C^{bl}(t)$ decays approximately monoexponentially and T exceeds a certain value a given concentration of indicator in brain at time T may arise from a relatively low blood flow which carries a relatively small amount of indicator to brain but provides basis for retention, *or* from a relatively high blood flow which carries a relatively high amount of indicator to the brain but results in a lower degree of retention However the particular shape of the curve of $C_{br}(T)$ as a function of p k and T (equation (10)) reveals that only one positive value of p satisfies equation (10) when

$$\frac{C_{br}(T)F^{bl}}{\lambda_{br}Q_a(T)} < \frac{k}{\exp[-k(T-t\ )]-1}$$

Thus the possibility of two solutions depends on the magnitude of T and the rate of decay of $C^{bl}(t)$ In Table 2 solutions to equation (10) for different values of k and T=20 s, and kt =0.32, are compared with value of f^{bl} calculated directly from equation (1), using E(T) values determined experimentally by collection of arterial and cerebral venous blood (cf equation (5)) For the values of k, T and kt chosen it is evident that only a single value satisfies equation (10)

Determination of blood-brain glucose transfer

Equations (1) (5) and (7) also apply to substances which penetrate into the extravascular compartment and only leve these by way of the venous blood The they also apply in the case of D-glucose which enters the brain by facilitated diffusion (Crone 1960). Following i v bolus injection of tracer glucose a certain quantity tracer is taken up by brain at the time T If no extracted tracer glucose has yet left the brain at that time (no back diffusion) and if no tracer glucose remains in the cerebral vessels, E(T) equals the unidirectionally extracted fraction of glucose in brain henceforth to be known as E_{br} is likely that E_{br}^{*} is constant for some time following injection and the large sink action of the cerebral extra and intracellular spaces for glucose (Bachelard et al 1970 Lund-Andersen & Kjeldsen 1977) cause labeled extracted glucose to escape from brain in the short period of 20 s (Betz et al. 1973)

If C_a^{pl} is the total concentration of glucose in arterial plasma equation (7) yields,

$$J_{br} = E_{br}\ f^{pl}C_a^{pl} = C^{pl}F^{pl}\ \frac{C_{br}^{*}(T)}{Q\ (T)}$$

where J_{br} represents the unidirectional blood brain glucose flux per unit weight of brain, $C_{br}^{*}(T)$ the extra[illegible] lar content of labeled glucose per unit weight of brain, Q (T) the quantity of labeled glucose collected in arterial sampling syringe Since glucose in plasma [illegible] brates ery slowly with glucose in erythrocytes [illegible] (Heath & Rose 1969) f^{pl} and F_a^{pl} refer to the respective plasma flow rates. The concentration C_a^{pl} does not [illegible] sent the concentration at which the transport occurs [illegible] different part of the capillary

It is important to emphasize that $C_{br}^{*}(T)$ must [illegible] no glucose in the vascular compartment of the brain [illegible] small amounts of labeled glucose were present in the [illegible] sel of the brain these amount were subtracted from contents of labeled glucose in brain in order to [illegible] $C_{br}^{*}(T)$ The amount of labeled glucose in the cerebral vessels per unit weight of brain at the time T [illegible] mated a $V^{pl}C_a^{pl*}(T)$ in which V^{pl} is the plasma [illegible] per unit weight of brai Thus $C_{br}^{*}(T)$ the amount labeled glucose located extravascularly in unit weight brain at time T was calculated a

$$(T) = C_b^*(T) - V^m C_p^{m*}(T) \quad (13)$$

which $C_b^*(T)$ is the total amount of labeled glucose in 1 ight of brain, V^m as equated with the mannitol ribution alone at time T obtained in separate expt described below mannitol was chosen because the 1 brain permeability is ery low (Lund-Andersen 9)

METHODS

of equation (10) for calculation of cerebral blood requires that the constants kt' and λ_{br} be know as since Q_{inject}, the injected dose of indicator F the of sampling by the arterial catheter $C_{ar}(T)$, the concentration of indicator per unit weight of brain, $Q_s(T)$, amount of indicator in the arterial sampling syringe (T), the concentration of indicator in arterial blood time T and T the sampling and decapitation time re determined experimentally Separate expt were formed to determine kt' and λ_{br}.

Estimation of validity of assumptions

Arterial concentration-time curves for butanol were obtained 3 rats by drawing arterial blood continuously into PP 50 catheter in which smearing was avoided by separation of individual samples by air bubbles The samples were transferred to counting vials and prepared as described below The maximal dilution of arterial plasma caused by the bolus injection as estimated from the peak activity of the arterial concentration time curves and the activity concentration in the injectate

The identity of concentration-time integrals obtained in the femoral and the left common carotid artery was examined in four rats following cannulation of the femoral artery and the left external carotid artery Ratios of time integrals in the three vessels were calculated.

The E(T) of butanol was measured directly according to equation (5) in 10 rats, using magnitudes of T ranging from 3 to 70 In order to avoid any interference of carotid cannulations with cerebral blood flow blood was drawn only from femoral artery and the superior sagittal sinus of the torcular in these expts.

Determination of k, t' λ_{br} (butanol), and V^m

The variables k and t' were determined in three rat by fitting a monoexponential decay curve to the experimentally determined values of $C_b^*(t)$ for butanol

We measured λ_{br} directly following prolonged equilibration of butanol in brain tissue in vivo. The samples of brain tissue corresponded to the samples used for blood flow determination and gave an estimate of the variation of λ_{br} between different regions The equilibration of butanol between brain and blood was performed as follows

In three rats, mixture of 15 µl 17µCi µl⁻¹ n-[1-¹⁴C]-butanol and 1500 µl unlabeled ethanol was slowly infused for 45 min, using fractionated infusion schedule designed to yield an approximately constant arterial concentration of butanol (Sokoloff et al The ethanol was included to exhaust the alcohol dehydrogenase system capacity for breakdown of alcohols during the expt and frequent arterial blood samples were taken to ensure the constancy of the arterial concentration At the end of the expt. the animal w decapitated, and samples of brain and arterial blood removed and assayed for radioactivity per unit weight of brain as described below The partition coefficient was calculated as the unit weight of brain to unit olume of blood radioactivity ratio

I 7 rats cerebral blood flow and cerebral content of labeled mannitol were determined simultaneously 70 s after bolus injection of a mixture of labeled butanol and labeled mannitol as described below The arterial plasma concentration of mannitol 20 after the injection was determined by measuring the mannitol concentration in the last portion of blood collected through the femoral arterial catheter in the manner described below V^m the plasma olume per unit eight of brain, was calculated a the ratio between $C_b^*(T)$ and $C_p^{m*}(T)$ for labeled mannitol

In the above derivation, it was assumed that mannitol does not cros the blood-brain barrier If mannitol does diffuse into the extravascular pace to some extent, the calculated plasma olume per unit weight of brain would include small apparent extravascular volume of distribution of mannitol. If labeled D-glucose diffuses passively across the blood-brain barrier to the same xtent, application of the mannitol distribution volume for V^m in calculation of $C_m^*(T)$ for labeled glucose will eliminate errors from passive blood-to-brain diffusion of D-glucose

Determination of regional erebral blood flow and blood-brain glucose transfer

In 5 rats, set of F F_f^* $C_{ar}(T)$ $C_b^*(T)$, $Q_s(T)$, $Q^*(T)$, C (T), Q_{inject}, $C_p^{m*}(T)$, and T were measured. $C_m^*(T)$ was calculated by means of equation (14). Then, regional and whole-brain blood-flow and regional and whole-brain blood-brain glucose transfer rates were calculated by iteration from equation (10) and (12) using values of kt' and λ_{br} obtained as mentioned above and calculating k by equation (11).

General procedure

The 350 g male Wistar rats were kept specific-pathogen-free with unlimited access to food and water Anesthesia was induced with ether and the animals were paralyzed with 3 mg kg suxamethonium, intubated and ventilated with 1% halothane vaporized in mixture of nitrogen and oxygen (70 30)

Catheters were placed in the tail artery both femoral arteries, and one femoral ein. A described above the superior sagittal sinus was cannulated in some rats. Cannulation of the superior sagittal sinus was performed in the following manner A small burr hole was placed immediately above the torcular care being taken not to rupture the dura. By means of micromanipulator capillary tube drawn out to fine tip was passed so gently through the dura that no leakage around the tube was observed. Arterial blood was assayed for pH P_{O_2} and P_{CO_2} with microelectrodes (Radiometer Copenhagen)

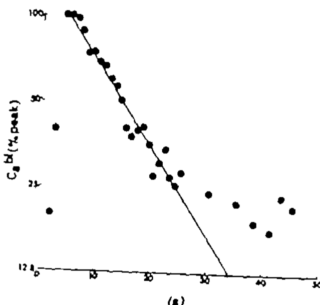


Fig 1 Typical arterial concentration curve (logarithmic ordinate scale) for butanol following an i v bolus injection of 200 μl Due to method of sampling peak activity was always represented in one of samples. Points regressed to equation $C_a^{bl}(t)=C^{bl}(4)\ \exp[-0.07(t-4)]$ the correlation coefficient r=0 98 in which is expressed in s Concentrations are relative to peak concentration

The animals were considered to be in respiratory steady state when Pa_{CO_2} in samples taken at least 10 min apart agreed within 10% Arterial blood pressure was continuously recorded with a strain gauge transducer

When the animals were in respiratory steady state with Pa_{CO_2} 30–35 mmHg Pa_{O_2} >100 mmHg and mean arterial blood pressure >100 mmHg a mixture of 5 μCi [14C]butanol and 20 μCi [3H]glucose in 200 μl Ringer HEPES buffer solution (Gjedde & Crone 1975) was injected into the femoral vein in less than a second Prior to mixing the [3H]glucose solution was air-dried and resuspended to eliminate volatile 3H-activity

Integration of concentration-time curves in blood was produced mechanically as described previously (Gjedde et al 1975) Briefly the samples were drawn into calibrated glass syringes at a known and constant rate during and for 20 s after i v injection At the end of 20s withdrawal was discontinued and the animal decapitated with a rodent guillotine The brain was rapidly removed and dissected bilaterally into the following regions weighing approximately 75 mg each Cerebellar hemisphere cerebellar vermis medulla and pons midbrain basal ganglia parietal cortex, and frontal occipital and temporal lobes

The blood and tissue samples were rapidly transferred to pre weighed counting vials containing 1.5 ml of a Soluene (Packard) and isopropanol mixture (1 1) The vials and samples were then reweighed blanched with 0.5 ml 35% hydrogen peroxide and stored overnight at 50 °C The next morning preparation was completed by addition of 20 ml of an Instagel (Packard) and 0.5 N HCl mixture (9 1). The vials were allowed to stabilize at counting temperature for 24 h prior to counting in a Tri-Carb 4.5 (Packard) liquid scintillation spectrometer (Hendler 1964)

Plasma concentrations of glucose were measured in freshly sampled arterial plasma by the glucose oxidase method (Christensen 1967 Hjelm & De Verdier 196) Arterial hematocrit, used for calculation of plasma concentrations of labeled glucose and mannitol from the respective whole blood activity concentrations, was determined by centrifugation.

Preparations of n-[1 14C]butanol, D-[1 3H(N)]glucose and D-[1 3H(N)]mannitol were obtained from New England Nuclear Corporation with specific activities of 1 Ci mmol 18 Ci mmol⁻¹ and 15–30 Ci mmol⁻¹ respectively

RESULTS

Determination of constants and confirmation of assumptions

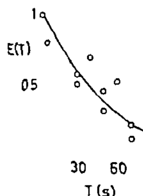
A representative arterial concentration-time curve for butanol is shown in Fig 1 The three curves obtained in this manner confirmed that the decay of butanol was approximately monoexponential from 4 to 20 s after the injection as required by equation (9) The variables k and t averaged 0.069±0.006 (S E n=3) and 4 7±0 7 s (S E n=3), respectively Multiplication of k and t yielded a value of of 0.32±0 06 (S E)

Integration of the curves revealed that the integral prior to peak time represented 10-15% of the total integral from 0 to 20 s The combined error made in the calculation of E(T) by assuming C^{bl} to be negligible for t<t as well as monoexponentially decaying for t≤t≤T was estimated as follows With the three arterial curves determined above E(T) was estimated numerically according to equation (8) using an average value for f^{bl}/λ_{br} of 0 028 s The values of E(T) calculated in this manner were compared with values of E(T) calculated according to equation (9) using the relevant values of k and the value of f^{bl}/λ_{br} used above The ratio of the former values of E(T) to the latter values of E(T) represents the factor by which blood flow wil

Table 3 *Integrals of arterial concentration time curves for butanol determined in right and left femoral arteries relative to integral determined in right common carotid artery*

Artery	Ratio* ± S.E (n=4)
Right femoral	0 99±0 03
Left femoral	1 0_±0 04

Curves were integrated for periods of 10-30 s after intravenous injection of a 200 μl bolus of labeled butanol



F 2 Directly measured net extractions of labeled ... from brain, E(T), vs. the time of measurement T. ... drawn ... result of least-squares computerized optimization of equation (9) to points shown. Curve corresponds to p=0.030 ... k=0.069 ... and t'=4.6 s.

... underestimated when calculated according to the ... described approximation procedures. This factor ... averaged 0.973±0.025 (S.E., n=3).

From the concentration of radioactivity in the ... injectate, the minimal dilution of the bolus was estimated to be 50-fold. Hence the glucose concentration in plasma was not significantly reduced by the bolus injection.

The amount of butanol measured in samples taken from each femoral artery relative to that measured in blood sampled from the right external ... artery is recorded in Table 3. The ratios confirmed that the arterial concentration-time curve integrals were equal in the three arteries for periods varying from 10 to 30 s after injection of the bolus.

E(T)-values of butanol directly measured for various values of T in ten rats are shown in Fig. 2. With k=0.069 s⁻¹ a least-squares computerized optimization (Gjedde & Crone 1975) of equation (9) to the experimental values of E(T) yielded a value of p of 0.030 s⁻¹. For λ_{br}=0.77 ml g⁻¹ this value corresponded to f^{br}=139 ml (100 g)⁻¹ min⁻¹. Using the E(T)-values and the corresponding measured C_a(T)-values for calculation of f^{br} from equation (1), the result came to 133±12 ml (100 g)⁻¹ min⁻¹ (±S.E.). As shown in Table 2, variation of k from 0.04 to 0.1 s⁻¹ did not significantly affect the close agreement.

The average brain-blood partition coefficients for 9 regions of brain are shown in Table 4. They did not significantly differ from the whole-brain average of 0.770±0.072 ml g⁻¹ (S.E.M.), indicating homogeneity of brain with respect to λ_{br}.

Table 4 *Brain-blood partition coefficients for butanol*

Region	λ_{br} (ml g⁻¹)
Cerebellar hemisphere	0.774 0.020
Cerebellar ... erm	0.774 0.032
Medulla and pons	0.777±0.032
Midbrain	0.744 0.026
Basal ganglia	0.746±0.033
Parietal cortex	0.780±0.040
Occipital lobe	0.819±0.063
Temporal lobe	0.775 0.037
Frontal lobe	0.791±0.038

±S.E. (3).

The plasma volume (mannitol distribution volume) per unit weight of whole-brain averaged 1.07±0.12 ml (100 g)⁻¹ (±S.E.) at a plasma flow of 56±9 ml (100 g)⁻¹ min⁻¹ corresponding to a mean transit time of 1.14 s. At the labeled glucose concentration in plasma at time T measured below these values corresponded to the presence in plasma of 8.6±0.. % of all labeled glucose in the brain.

Determination of regional cerebral blood flow and blood bra glucose t ansf

The mean arterial blood pressure, blood gases, pH hematocrit, arterial plasma glucose concentration and cardiac output of five rats are shown in Table 5.

Table 5 *Blood pressure blood gases pH hematocrit arterial plasma gluco and ardiac output of rats used i the present study*

Variable	Mean±S.E. (=5)
MABP (mmHg)ᵃ	100±3
Pa_{O_2} (mmHg)ᵇ	130±15
Pa_{CO_2} (mmHg)ᶜ	33±2
pH_aᵈ	7.47±0.02
hctᵉ	0.44±0.02
C_a^g (mmol l⁻¹)ᶠ	10.4±0.9
F_{heart} (ml kg⁻¹ min⁻¹)ᵍ	139±5

ᵃ Mean arterial blood pressure. ᵇ Arterial oxygen tension. ᶜ Arterial carbon dioxide tension. ᵈ Arterial pH. ᵉ Hematocrit. ᶠ Arterial plasma glucose concentration. ᵍ Cardiac output was determined by extrapolation of the monoexponential arterial decay curve beyond the time of onset of recirculation. Thus if F_{heart} is the cardiac output and Q_{total} the total administered dose of labeled butanol, for t≥t', $Q_{total}=F_{heart}^{bl}\int_{t'}^{\infty}C_a^{bl}(t')\exp[-k(t-t')]dt$. Since $Q_a(T)=F^{bl}\int_{t'}^{T}C_a^{bl}(t)\exp[-k(t-t')]dt$, then, by division, $F_{heart}=[1-\exp(kt'-kT)]F_a^{bl}Q_{total}/Q_a(T)$, from which equation cardiac output was calculated when k was known.

Table 6 *Whole-brain blood flow blood-brain glucose flux and tracer glucose extraction*

Variable	Mean±S E (n=5)
f^{bt} (ml (100 g)$^{-1}$ min^{-1})^a	179± 7
J_{glc} (μmol (100 g)$^{-1}$ min^{-1})^b	144±13
E_{glc}	0 1± 0 01

Whole brain blood flow ^{b} Blood-brain glucose flux Tracer glucose extraction

In these rats the following values for butanol and labeled glucose were determined at T=20 s (X± S E) F^{bl}=0 0912±0 0016 ml min $Q_a(T)$=2 60± 0 11 nCi $C_{br}(T)$=2 94±0 22 μCi (100 g)$^{-1}$ Q (T)= 18 7±0 7 nCi $C^*_{br}(T)$=6 07±0 45 μCi (100 g) $C^{bl}(T)$=74±6 nCi ml $C^{bl*}_{p}(T)$=500±67 nCi ml and Q_{total}=7 1±0 5 μCi kg^{-1}

Using kt =0 32 the variable k was calculated from equation (11) to be 0 058±0 008 s

With kt =0 32 λ_{br}=0 77 ml g and k=0 058 s^{-1} equation (10) was used to estimate cerebral blood flow by iteration Blood brain glucose flux was calculated by means of equation (12)

Whole-brain blood flow and unidirectional blood-brain glucose flux are recorded in Table 6

The distribution of blood flow and blood-brain glucose transfer rates per 100 g of tissue in nine regions relative to the whole-brain average are shown in Fig 4 Gross regional perfusion coefficients were highest in the midbrain and lowest in the cerebellar hemispheres and frontal lobe Blood-brain glucose flux was highest in the parietal cortex the only purely cortical region sampled

DISCUSSION

The present study shows that it is possible to measure regional cerebral blood flow and blood brain glucose transfer simultaneously by a rapid experimental procedure

The whole-brain blood flow rate measured here agrees with values obtained by Sakurada et al (1978) and is approximately 30% higher than the value reported by Gjedde et al (1975)

The unidirectional blood-brain glucose transfer observed in this study is twice as high as values for the rat published by Bachelard et al (1973) and Pardridge and Oldendorf (1977)

Cerebral blood flow

The present blood flow method resembles the microsphere technique but has the advantage that the indicator can be injected i.v., i e with minimal circulatory perturbation (Gjedde et al. 1977). The microcirculation of the brain is not affected.

The assumption that brain uptake of butanol is entirely flow-limited at the flow rates encountered in the present study was confirmed by the observation of good agreement between the value of k estimated from data for a single bolus passage of butanol and the value determined directly by equilibration between blood and brain tissue The butanol is suitable for measurements of f^{bl} of magnitudes about 100 ml (100 g) min Raichle et al (1976) arrived at the same conclusion at somewhat lower cerebral blood flow rates in the monkey

The whole brain blood flow measured here applies to rats in halothane anesthesia, a condition known to increase cerebral blood flow (Gjedde & Hindfelt 1975)

Van Uitert & Levy (1978) used the same method to measure regional cerebral blood flow and CO_2-response in the awake gerbil They decapitated 10 s after the i v injection E(T) was found to be at least 95% in normal gerbils and was consequently considered to be unity Since E(T) decreases with increasing flow it is likely that the CO_2-response was underestimated

The regional distribution of blood flow rates showed only moderate variations. The highest

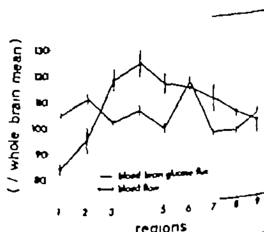


Fig 3 Gross regional brain blood flow and blood brain glucose transfer rates in rat expressed relative to whole brain means given in Table 5 Regions represent 1 cerebellar hemisphere cerebellar vermi 3 medulla and pons 4 midbrain 5 basal ganglia 6, parietal cortex 7 occipital lobe 8, temporal lobe 9 frontal lobe Bars represent S E.

rebral blood flow was observed in the midbrain, accordance with results published by Sakurada et (1978)

and brain glucose transfer

The present method resembles the method used by Bachelard et al. (1973) but is a single injection rather than a constant infusion experiment. The present protocol eliminated several disadvantages. In the study of Bachelard et al., the arterial glucose concentration was kept constant for 3 minutes, during which period unknown amounts of radioactivity escaped from brain.

Single carotid injections performed rapidly as required by the Oldendorf technique (Pardridge & Oldendorf 1977), cause unknown increases of arterial blood pressure that may exceed the upper pressure limit of autoregulation and cause temporary increases of cerebral blood flow.

Previous values for glucose transport were measured in rats anesthetized with barbiturate or other drugs known to reduce cerebral blood flow and possibly also the unidirectional glucose flux by a reduction of the capillary surface area available for blood-brain glucose transport.

The rate of glucose transport into brain observed in the present study agrees with the rate of uptake of [ ]-deoxy-D-glucose in brain of awake rat, reported by Sokoloff et al. (1977).

In conclusion, it is possible to measure cerebral blood flow and blood-brain glucose transfer rates by a short-lasting procedure. The short time is important to studies of the regulation of cerebral metabolism. The rates of blood flow and glucose supply are essential to the metabolism of the brain because increases of cerebral metabolic rate depend on adequate delivery of metabolic substrates. Thus in studies of cerebral metabolism measurements of blood flow and glucose supply must be short enough to detect rapidly changing functional states.

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Rapid steady state analysis of blood brain glucose transfer in rat

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GJEDDE, A Rapid steady-state analysis of blood-brain glucose transfer in rat Acta Physiol Scand 1980 108.331 339 Received 17 Jan. 1979 ISSN 0001-6772 Institute of Medical Physiology Dept. A University of Copenhagen, Denmark.

A new kinetic analysis of blood-brain glucose transport is described based on steady state model that takes account of cerebral blood flow mean capillary glucose concentration, and cerebral metabolic rate. The maximal rate (T_{max}) and half-saturation constant (K_m) of glucose transport from blood to brain were determined in rats by measuring the rate of blood-to-brain glucose transfer at different blood glucose concentrations Each determination lasted 20 seconds. For whole-brain, T_{max} and K_m averaged 258±33 (S E.) μmol (100 g)$^{-1}$ min and 5 9±1 6 (S E) mmol l respectively The regional variations were insignificant. The new approach permits kinetic parameters to be measured locally in brain in rapidly changing functional states.

Key word D-glucose blood-brain barrier facilitated diffusion hyperglycemia, hypoglycemia

Glucose, the major nutrient of brain passes from blood to brain by facilitated diffusion (Crone 1960). Impaired blood-brain glucose transport has attracted attention as a possible contribution to cerebral metabolic dysfunction in hepatic encephalopathy (Cremer et al. 1975), Reye s syndrome (Haymond et al. 1978), and salicylism (Thurston et al 1970)

Kinetic analysis of blood-brain glucose transfer requires knowledge of blood flow plasma glucose concentration profile in cerebral capillaries and brain extracellular glucose concentration. Optimally these variables should be determined simultaneously but previous kinetic analyses in rat have suffered from incomplete knowledge of one or more of the variables (Bachelard et al. 1973 Brender et al 1975 Betchiazzo et al. 1970 Oldendorf 1971). Either blood flow was left undetermined or average capillary glucose concentration was equated with the arterial glucose concentration The glucose concentration in brain extracellular water is low (A. Gjedde, A. J Hansen & I A Silver unpublished) but the effect of different concentrations is relatively unknown.

A tracer method has been developed which allows the simultaneous determination of cerebral blood flow (whole-brain and regional) and unidirectional blood-brain glucose transfer by a procedure lasting 20 s. Normal values for rat were presented previously (Gjedde et al 1980).

In the present study the steady-state glucose concentration in arterial blood was varied by administration of insulin or glucose and the rates of blood-brain glucose transfer at different concentrations were used to calculate the kinetic constants for the facilitated diffusion of glucose from blood to brain. Calculation of the kinetic constants was based on mathematical treatment of the conventional single-capillary model of the cerebral circulation

METHODS

Briefly bolus injections of a mixture of labeled butanol and tracer glucose were given in the femoral vein of rats. Arterial blood was slowly withdrawn into a syringe for 20 s. At the end of the collection the rats were decapitated and the brain rapidly removed for further processing

Theory

Uptake of radiolabeled glucose by brain following an intravenous bolus injection may be treated mathematically in the manner described by Bradbury et al. (1975) for tracers that enter brain by simple diffusion, and by Bass et al. (1976) for the enzymatic elimination of galactose in liver Both approaches treat single capillary situation.

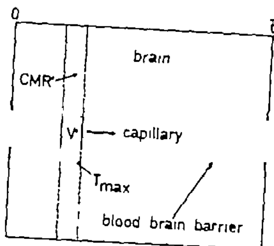


Fig 1 Schematic representation of single-capillary model of brain used to treat blood brain glucose transport mathematically The volume V enters at time 0 and exits at time t Assumptions about the model are explained in the text

Assume that backflux of labeled glucose from brain to capillary is negligible within 20 s that axial diffusion within the capillary is negligible that there is complete cross-stream mixing within the capillary i e Taylor effects (Lassen & Crone 1970) are not too important that a single "average" capillary with surrounding coaxial cylindrical tissue mantle can represent the brain as a whole that the animal is in a steady-state with respect to the concentration of glucose in arterial plasma and the plasma flow rate of the brain and that erythrocytes do not exchange glucose with plasma Further assume that glucose is transported from blood to brain through a functionally single barrier by means of a symmetrical carrier that the glucose transport capacity and the tissue rate of glucose metabolism are uniformly distributed along the length of the capillary and that the simple diffusion permeability of the blood brain barrier for glucose is negligible (Lund Andersen 1979).

Consider an "average" capillary of length L with surrounding tissue (Fig. 1) Let C_a^{pl} and C_a^{pl*} represent the plasma concentrations of unlabeled and labeled glucose respectively in the arterial end of the capillary Following a bolus injection of tracer into a distant vein a minute fraction of the bolus will reach the capillary Let V be so small a volume of plasma (containing a fraction of the bolus that passes through the capillary) that longitudinal concentration gradients within V are negligible Throughout its passage V is in contact with a capillary surface with a glucose transport capacity T_{max} and supplies a volume of tissue with a rate of glucose metabolism CMR_{glc} Accordingly

$$\frac{V'}{V} = \frac{T'_{max}}{T_{max}} = \frac{CMR'_{glc}}{CMR_{glc}}$$

where V T_{max} and CMR_{glc} represent capillary plasma volume glucose transport capacity and glucose consumption per unit weight of whole-brain or a specified part of brain The linear velocity of V equals $f^{pl}/(nA)$ where f^{pl} is the plasma flow and n the number of capillaries per unit weight of brain and A the average plasma cross-sectional area of brain capillaries. The plasma transit time t can be expressed as

$$t = nAL/f^{pl} = V/f^{pl}$$

By the definitions given above

$$T'_{max}/V' = T_{max}/V = a$$

and

$$CMR'_{glc}/V' = CMR_{glc}/V = b$$

Provided T'_{max} and K_m are independent of C the local concentration of glucose in brain extracellular fluid the following equations relate the movement of labeled and unlabeled glucose from blood to brain during the passage of the bolus containing labeled and unlabeled glucose (Wilbrandt & Rosenberg 1961)

$$V' \, dC^{pl*}/dt = -T'_{max} C^{pl*} /(K_m + C^{pl})$$

and

$$V' \, dC^{pl}/dt = -T'_{max} \left(\frac{C^{pl}}{K_m + C^{pl}} - \frac{C_i}{K_m + C_i} \right)$$

in which K_m is the half saturation constant of the facilitated diffusion process C^{pl} the concentration of glucose in the volume V and C^{pl*} the concentration of labeled glucose in V both functions of time spent in the capillary The provision that T'_{max} and K_m be constant will be discussed below T_{max} and K_m are operational constants borrowed from enzymology and used for the experimentally derived maximal rate and half-saturation concentration of the transendothelial transfer mechanism in steady state The local consumption of glucose equals the difference between inward and outward fluxes of unlabeled glucose across the blood-brain barrier Thus assuming CMR_{glc} to be the same at all points along the capillary

$$V' \, dC^{pl}/dt = -CMR'_{glc} \quad (3)$$

After substitution of $CMR'_{glc} = V' \, b$ and integration from $[0 \;\; C_a^{pl}(0)]$ to $[t \;\; C^{pl}(t)]$

$$C^{pl} = C_a^{pl} - bt \quad (4)$$

This result expresses the fact inherent in the assumptions that unlabeled glucose falls linearly from the arterial to the venous end of the capillary in the steady-state

By insertion of (4) in (1)

$$\frac{dC^{pl*}}{C^{pl*}} = \frac{-a \, dt}{K_m + C_a^{pl} - bt} \quad (5)$$

and by integration of (5) from $[0 \;\; C^{pl*}(0)]$ to $[t \;\; C^{pl*}(t)]$

$$\ln \left[\frac{C_v^{pl*}}{C_a^{pl*}} \right] = \frac{a}{b} \ln \left[\frac{K_m + C_a^{pl} - bt}{K_m + C_a^{pl}} \right] \quad (6)$$

hich C_i^*/C_i^{**} represents the transmitted fraction f labeled glucose. After substitution of t, a, and b, and rearrangement,

$$E_{ae} = 1 - \exp\left[\frac{T_{max}}{CMR_{glc}} \ln\left(1 - \frac{CMR_{glc}}{f^{pl}(K_m + C_p^a)}\right)\right] \quad (7)$$

hich E_{ae}^* ... the extracted fraction of tracer glucose when no tracer glucose enters V ... from the brain during its passage through the capillary. Equation (7) ... estimation of the relationship between T_{max} and K_m ... sets of E_{ae}^*, CMR_{glc}, f^{pl} and C_p^a are known. If the ratio $CMR_{glc}/[f^{pl}(K_m + C_p^a)]$ does not exceed 0.1 as ... below to be the case, equation (7) can be simplified to exclude CMR_{glc}:

$$E_{ae} = 1 - \exp\left[\frac{T_{max}}{f^{pl}(K_m + C_p^a)}\right] \quad (8)$$

Equation (5) shows that the concentration of labeled glucose has an almost monoexponential fall along the capillary, and thus has ... profile along the capillary different ... that of unlabeled glucose.

Corresponding values of T_{max} and K_m can be determined after variation of C_p^a or f^{pl} provided T_{max} is constant. Equation (8) is akin to the equation used by Crone (1963) to determine simple diffusion permeabilities for ... by the indicator diffusion method: $E_{ae} = 1 - \exp[-PS/f^{pl}]$ ... here PS corresponds to $T_{max}/(K_m + C_p^a)$. The equation shows that the decrease ... plasma glucose concentration as blood passes along the capillary has little influence on the magnitude of the calculated kinetic constants when the ratio $CMR_{glc}/[f^{pl}(K_m + C_p^a)]$ ... 0.1. When equation (8) applies, ... plot of $f^{pl} \ln(1 - E_{ae})$ versus $-C_p^a f^{pl} \ln(1 - E_{ae})$ fits a straight line with the slope K_m and ordinate intersection T_{max}.

Measurement of cerebral blood flow and blood brain glucose transfer

Whole-brain and regional cerebral plasma flow rates, f^{pl}, were calculated from tissue and arterial blood sample contents of radioactive butanol, using the equation,

$$f^{pl} = \frac{1}{E(T)} F_p^a \frac{C_B(T)}{Q_a(T)} \quad (9)$$

in which E(T) ... the net cerebral extraction fraction of radioactive butanol at the time T after the introduction of the indicator into the circulation, F_p^a the constant rate of collection of arterial plasma, $Q_a(T)$ the amount of radioactive butanol collected, and $C_B(T)$ the amount of indicator per unit weight of a sample of brain to which f^{pl} refers. E(T) was estimated as described previously (Gjedde et al. 1980).

Whole-brain and regional blood-brain glucose transfer rates were calculated from tissue and arterial blood sample contents of radioactive glucose, assuming no loss of labeled glucose from the brain during the time T (Gjedde et al. 1980),

$$J_{ae} = C_p^a F_p^a \frac{C_B^*(T)}{Q_a^*(T)} - C_p^a f^{pl} \left[\frac{C_B^*(T) - V^{pl} C_p^{a*}(T)}{Q_a^*(T)}\right] \quad (10)$$

where J_{ae} represent ... the unidirectional blood-brain glucose flo... per unit weight of brain, C_p^a the arterial plasma glucose concentration, f^{pl} the plasma flow per unit weight of the sample of brain, F_p^a the ... olume of plasma collected in the arterial sampling ... yringe in unit time, $C_{ev}^*(T)$ and $C_B^*(T)$ the extravascular and total contents of labeled glucose per unit weight of the sample of brain, V^{pl} the plasma ... olume in brain, $Q_a^*(T)$ the total content of labelled glucose in the arterial sample, and $C_p^{a*}(T)$ the concentration of labeled glucose in arterial plasma at the time T. The plasma ... olume was measured in separate experiments as described previously (Gjedde et al. 1980).

The e... traction fraction of tracer glucose was calculated from ... rearrangement of equation (9),

$$E_{ae} = \frac{F_p^a C_{ev}^*(T)}{f^{pl} Q^*(T)} \quad (11)$$

General procedure

12 male Wistar rat... were divided in 3 groups. Rat... in the *normal glucose* group received no treatment. Rat... in the *low glucose* group received 2 IU kg ... insulin neutral (Leo) ... p. 1 h prior to study. Rats in the *high glucose* group received 17 mmol kg ... D-glucose i.p. 1 h prior to study.

The 300–350 g rats were kept specific-pathogen-free and fed food and water ad libitum. Anesthesia was induced with ether, and the animals were paralyzed with 3 mg kg^{-1} suxamethonium, intubated and ventilated with 1% halothane vaporized in ... mixture of nitrogen and oxygen (70 : 30).

Catheters were placed in the tail artery, both femoral arteries, one femoral vein, and the superior sagittal sinus of the brain. Cannulation of the superior sagittal sinus was performed as described previously (Gjedde et al. 1980).

Arterial blood samples were assayed for pH, P_{O_2} and P_{CO_2} with microelectrodes (Radiometer, Copenhagen). The animals were considered to be in respiratory steady-state when P_{aCO_2} in samples taken at least 10 min apart agreed within 10%. Arterial blood pressure was continuously recorded with ... strain gauge transducer.

When the animals were in respiratory steady-state ... mixture of 5 µCi [^{14}C]butanol and 20 µCi [^{3}H]glucose in 200 µl Ringer HEPES buffer solution (Gjedde & Crone 1975) was rapidly injected into the femoral vein. Prior to mixing, the [^{3}H]glucose solution was air-dried and resuspended to eliminate all volatile ^{3}H-activity.

Integration of concentration-time curves of arterial blood were produced mechanically as described previously (Gjedde et al. 1980). Briefly, arterial blood was drawn into calibrated glass syringes at ... known and constant rate during and for 20 ... after injection. At the end of 20 s, withdrawal was discontinued and the animal decapitated with ... rodent guillotine. The brain was rapidly removed and dissected bilaterally into the following regions, weighing approximately 75 mg each: Cerebellar hemisphere, cerebellar vermis, medulla and pons, midbrain, basal

Table 1 *General conditions of the three groups of rats used in the present study*

Group	Normal glucose (n=5)	High glucose (n=4)	Low glucose (n=4)
MABP (mmHg)[a]	100±3	102±13	101±8
hct (%)[b]	44±2	48±?	44±
Pa_{CO_2} (mmHg)[c]	33±2	29±0 4	35±2
Pa_{O_2} (mmHg)[d]	130±15	145±20	14?±20
pH[e]	7 47±0 02	7 41±0 03	7 39±0 02
f^{pl} (ml (100 g) min)	72±4	45±10	65±7

Values indicate mean ± S E Number of observations in brackets

Mean arterial blood pressure [b] Arterial hematocrit Arterial carbon dioxide tension [d] Arterial oxygen tension Arterial pH [f] Cerebral plasma flow rate

ganglia parietal cortex and frontal occipital and temporal lobes.

The blood and tissue samples were rapidly transferred to pre weighed counting vials containing 1.5 ml of a Soluene (Packard) and isopropanol mixture (1 1) The vials and samples were then re-weighed blanched with 0.5 ml 35% hydrogen peroxide and stored overnight at 50°C The next morning preparation was completed by addition of 20 ml of an Instagel (Packard) and 0 5 N HCl mixture (9 1) The vials were allowed to stabilize at counting temperature for 4 h prior to counting in a Tri-Carb 2425 (Packard) liquid scintillation spectrometer

Plasma concentrations of glucose were measured in freshly sampled arterial and cerebral venous plasma by the glucose oxidase method (Christensen 1967 Hjelm & De Verdier 1963) Blood concentrations of oxygen were determined by carbon monoxide displacement in a galvanic fuel cell (Lex-O_2-Con Lexington Instruments) Arterial hematocrit wa determined by centrifugation

Consumption of glucose and oxygen was calculated by multiplying arteriovenous deficits with the whole brain plasma and blood flow rates respectively

Preparations of n-[1 ^{14}C]butanol D-[1 H(N)]glucose and D-[1 H(N)] mannitol were obtained from the New England Nuclear Corporation with specific activities of 1 86 mCi mmol 18 Ci mmol and 15–30 Ci mmol respectively

RESULTS

The average whole brain plasma flow rates of the three groups of rats arterial blood gases pH and mean blood pressure are recorded in Table 1 No significant differences were noted between the three groups

The average plasma volume (measured with mannitol) per unit weight of brain of the three groups of rats were 1 01±0 1? (S E. n=?) 0.9? 0 11 (S E n=4) and 0.54±0 03 (S E., n=4) ml (100 g)$^{-1}$ respectively in normo- hyper and hypoglycemic animals

The observed arterial glucose and oxygen concentrations and the cerebral metabolic rates of glucose and oxygen are shown in Table 2. Glucose consumption calculated as f^{pl} ($C_a^{pl}-\bar{C}_v^{pl}$) was insignificantly increased in hyperglycemia and markedly reduced in hypoglycemia and markedly reduced in hypoglycemia Oxygen consumption was similar in the three groups Oxygen consumption in the normo- and hyperglycemic groups reflected the normal ratio (6 mol/mol) between oxygen and glucose consumption in the brain In the hypoglycemic group oxygen consumption exceeded the rate calculated stoichiometrically from glucose consumption indicating oxidation of other substrates than glucose as also noted by Norberg & Siesjö (1976)

The average whole brain extraction fraction and flux of unidirectionally transported glucose of the three groups are shown in Table 3 The relationship between the concentration of glucose in arterial plasma and the transport of glucose from blood to brain in individual animals is shown in Fig 2. It is evident that the unidirectional blood-brain glucose transfer increases at a declining rate with increasing plasma glucose concentration This phenomenon known as saturation is one of several criteria of facilitated diffusion and forms the basis for the use of the Michaelis-Menten formalism that leads to a maximal transport rate and a half saturation constant For reasons outlined in theory above it is meaningless to calculate a regression curve between arterial glucose concentration and blood-brain glucose transfer for the points shown in Fig 2 because the blood-brain glucose flux is not a function of the arterial plasma concentration alone It was shown that the glucose metabolic rate can be ignored in the calculation of j_{gluc} when the ratio $CMR_{glc}/[f^{pl}(K_m+C_a^{pl})]$ does not exceed 0.1 (cf Table 2) A plot of $-C_a^{pl}\, f^{pl} \ln(1-E_{glc})$ against $f^{pl} \ln(1-E_{glc})$ yields T_{max} and K_m (cf Fig 3) The points regressed to the linear equation y=-59x -58 with a correlation coefficient of 0 7 indicating a T_m of 258±33 (S E) μmol (100 g) min and a K_m of 5 9±1 6 (S E) mmol l

The regional variation of T_{max} and K_m was investig

[illegible] 2 Arterial concentrations and cerebral metabolic rate for oxygen and glucose

[illegible]	Ca_{O_2} (mmol l^{-1})^a	Ca (mmol l^{-1})^b	CMR_{O_2} ($\frac{\mu mol}{100 g min}$)^c	CMR_{glc} ($\frac{\mu mol}{100 g min}$)^d	$\frac{CMR_{glc}}{f^{wb}(C_a^{pl} K_m)}$
[illegible] glucose	9 1±0 1	10 4 0 9	379 49	67_10	0 0571
[illegible] glucose	10 4±0	30 8±0 7	433_76	74± 8	0 0448
[illegible] glucose	9 1+0 7	1.8_0 6	313_63	31 6	0 0619

[illegible] indicate mean ± S E.

[illegible] oxygen concentration (whole-blood) Arterial plasma glucose concentration Whole-brain metabolic rate [illegible] oxygen Whole-brain metabolic rate for glucose

[illegible] and indicated homogenous tissue No [illegible] white matter was sampled however

[DI]SCUSSION

[Th]e present study revealed a maximal rate of trans[port] of glucose across the blood-brain barrier higher [th]an two previous estimates in rat (Brender et al [19]75 Pardridge & Oldendorf 1977). The observed [ap]parent affinity constant is only slightly lower than [fr]om estimates in rat (Bachelard et al. 1973 [Bre]nder et al 1975 Buschiazzo et al. 1970 Pard[rid]ge & Oldendorf 1977)

[Max]imal transport rate

[Th]e transport capacity of the blood-brain barrier [fo]r glucose is affected by a number of factors that [ar]e at present not well known It is not unlikely [th]at the maximal transport capacity is related to the [sur]face area of perfused capillaries which may vary [be]tween species, and in a particular animal under [va]rious conditions. It is possible therefore that the assumption of constant T_{max} in the range of plasma glucose concentration from 1.8 to 30 mM is not valid In particular the question may be raised whether the low blood flow rate (f^{wb}) observed in the hyperglycemic rat were not associated with a reduction of the number of perfused capillaries and thus of T_{max} However the fact that E_{glc} was not found to be much lower in the hyperglycemic animals than in the normoglycemic speaks against this possibility An elevation of C_a^{pl} and a reduction of T_{max} should both (cf equation (7) lead to a decrease of E_{glc} It is therefore likely that the lower f^{wb} has exerted the opposite effect on E_{glc} as it would when the number of perfused capillaries (and T_{max}) was unchanged and the plasma transit time prolonged.

Other factors which may affect blood-brain glucose transport include anesthetic agents They may act directly on the endothelial membranes or indirectly through changes of cerebral blood flow Thus, Mayman et al (1964) and Alexander et al.

[Ta]ble 3. *Blood-brain glucose transport in the three [gro]ups of rats*

Group	E_{glc} (%)	J_{glc} (µmol (100 g)$^{-1}$ min^{-1})
Normal glucose	21±1	150±11
High glucose	17±3	205±27
Low glucose	37±1	44±13

[Val]ues indicate mean ± S E

Extracted fraction of unidirectionally transported tracer [gl]ucose. Blood-brain glucose flux per unit weight of whole brain

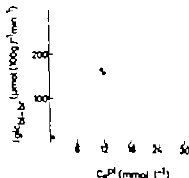


Fig 2 Relationship between arterial plasma glucose concentration and unidirectional blood-brain glucose flux per unit weight of whole-brain in rats rendered normo- hypo- or hyperglycemic by administration of glucose or insulin.

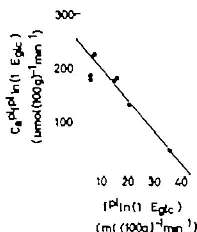


Fig 3 Estimate of the constants T_{max} and K_m for the transport of glucose from blood to brain by means of a double-logarithmic plot based on the mathematical treatment of blood-brain glucose transfer developed in the text

(1975) reported that halothane and pentobarbital may render the blood brain barrier more permeable to hexoses In most of the previous studies on rat cited above anesthesia was achieved by barbiturates while halothane was used in the present study For this reason alone direct comparison with the present study is difficult Furthermore the value for maximal glucose transport reported by Pardridge and Oldendorf (1977) was not based on measurement of cerebral blood flow although this variable was needed to calculate transport rates (Gjedde & Crone 1975)

Half saturation constant

The half saturation constant measured in the present study ranks among the lower ones in the literature and was determined with correction for passive diffusion The determination of the half saturation constant is very sensitive to uncertainties about the exact capillary glucose concentration Therefore steady state with respect to unlabeled glucose concentrations is a prerequisite for accurate calculation of the constant non steady state may introduce marked redistribution of unlabeled glucose between blood and brain (Lund Andersen & Christensen 1979)

Compartmental analysis

A compartmental analysis of blood-brain glucose transfer and phosphorylation rates following i v injections of tracers was performed by Buschiazzo et al (1970) No account was taken of the average glucose concentration in the cerebral capillaries and the extracellular glucose concentration was assumed to approach the mean capillary plasma concentration at high glucose levels in blood. Equal concentrations have never been observed in experiments nor can they be inferred from the symmetrical nature of the glucose carrier Equation (...) in fact predicts that provided CMR_{glc} is constant, the plasma to brain glucose ratio is rather constant at plasma glucose concentrations above a certain level (of the order of 20 mM) falling only when the concentration reached very high levels. For a considerable range of plasma concentrations, the major determinant of the concentration ratio is the CMR_{glc}

A kinetic analysis of brain glucose uptake was performed by Pappenheimer & Setchell (1973) in sheep and rabbit Their analysis was based on the assumption that the difference between capillary and brain glucose concentrations falls exponentially along the capillary reaching zero asymptotically and that the brain extravascular glucose concentrations in the steady state can be represented by the glucose concentration in cerebrospinal fluid. It was suggested in the present analysis that the capillary glucose concentration more likely falls linearly at a rate determined by the glucose consumption of the tissue It was recently convincingly argued by Lund Andersen (1979) that free glucose is distributed uniformly in brain water and that its concentration reaches no more than half of that in cerebrospinal fluid Pappenheimer & Setchell however obtained values for maximal glucose transport and half saturation constant in the sheep and rabbit which agree with the results of the present study

It is important to note that the present model requires that the metabolic rate for glucose in brain be independent of the glucose concentration in blood and brain Although this was not the case in the present study it was shown that CMR_{glc} did not enter into the calculation of the kinetic constants from data from the entire range of concentrations studied

However in hypoglycemia in which low plasma glucose levels directly or indirectly cause CMR_{glc} to fall the assumption made here in integrating equation (1) i e that CMR_{glc} be the same at all points along the capillary may no longer be fulfilled and equations (6) (7) and (8) therefore not valid for the case of hypoglycemia. The weight of this objection depends on the influence of a varied CMR_{glc} on the me ... E_{glc} In the hypo

...ycmic animals, the net cerebral glucose extraction fraction is about 25% indicating a fall of unlabeled glucose concentration of the same magnitude during the passage of blood through the cerebral capillaries. If CMR_{glc} decreased by the same amount, i.e. from 35 µmol (100 g) min at the arterial end of the capillary to 27 µmol (100 g) ... at the venous end of the capillary (in order to yield an average CMR_{glc} per unit weight of whole brain of 31 µmol (100 g)$^{-1}$ min) stepwise integration of equation (5) (e.g. by division of the capillary into ten equal sections in which CMR_{glc} decreased linearly from one section to the next) predicts that the resulting E^*_{glc} would decrease by less than 1% ... the value calculated from equation (7) under the assumption that CMR_{glc} remained constant and equal to the CMR_{glc} present at the arterial end of the capillary. The assumption of constant CMR_{glc} therefore fails to introduce any significant error in the final estimation of T_{max} and K_m from values of E^*_{glc} obtained in the entire range of C^a_p values studied.

The present study took account of cerebral blood flow, the cerebral metabolic rate for glucose and the capillary concentrations of labeled and unlabeled glucose. It was a consequence of the model that labeled and unlabeled glucose appeared to be ... differently, unlabeled glucose falling linearly and labeled glucose falling exponentially in the capillary. No account was taken of the possibility that the blood-brain barrier may consist of two ... functionally distinct barriers represented by the luminal and abluminal membranes of the cerebral capillary endothelial cell. The analysis yielded ... under rate constants for the blood-brain transport of glucose, based on a model which included more of the variables that effect the measurement of blood brain glucose transport than have previous studies.

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LIST OF SYMBOLS

Symbol	Description
...	average plasma cross-sectional area of single brain capillary (cm²)
...	ratio between maximal rate of unidirectional blood-brain glucose transport and capillary plasma volume per unit weight of brain (mM min)
b	ratio between glucose utilization and capillary plasma volume per unit weight of brain (mM min)
C^{a}	total D-glucose concentration in arterial plasma (mM)
$C^{a*}_p(t)$	concentration of labeled D-glucose in arterial plasma as function of time after introduction into the circulation (nCi ml)
$C^{a*}_p(T)$	concentration of labeled D-glucose in arterial plasma at time of decapitation (nCi ml)
$C^{c}( )$	total concentration of glucose in capillary plasma as function of time spent in the capillary (mM)
$C^{c*}( )$	concentration of labeled D-glucose in capillary plasma as function of time spent in the capillary (nCi ml)
C^{v}	total concentration of D-glucose in venous plasma (mM)
$C^{v*}(t)$	concentration of labeled D-glucose in venous plasma as function of time after introduction into the circulation (nCi ml)
C	total concentration of D-glucose in extracellular water in brain (mM)
$C_{b}(T)$	content of labeled D-glucose per unit weight of brain at time of decapitation (nCi (100 g))
$C^*_{ev}(T)$	extravascular content of labeled D-glucose per unit weight of brain at time of decapitation (nCi (100 g))
$C^{a}(t)$	concentration of labeled butanol in arterial blood as function of time after introduction into the circulation (nCi ml)
$C^{a}(T)$	concentration of labeled butanol in arterial blood at time of decapitation (nCi ml)
$C_{br}(t)$	content of labeled butanol per unit weight of brain as function of time after introduction into the circulation (nCi (100 g)$^{-1}$)
$C_{br}(T)$	content of labeled butanol per unit weight of brain at time of decapitation (nCi (100 g))
$C^{v}(t)$	concentration of labeled butanol in cerebral mixed venous blood as function of time after introduction into the circulation (nCi ml^{-1})
$C_{i}(T)$	content of labeled butanol per unit weight of an organ indicated by subscript at the time of decapitation (nCi (100 g))
Ca_{O_2}	concentration of oxygen in arterial blood (mmol l)
CMR_{glc}	metabolic rate for glucose per unit weight of brain (µmol (100 g)$^{-1}$ min^{-1})
CMR_{glc}	metabolic rate of glucose in small section of average single capillary (µmol min^{-1})
CMR_{O_2}	metabolic rate for oxygen per unit weight of brain (µmol (100 g)$^{-1}$ min^{-1})
E_{glc}	fraction of unidirectionally extracted glucose in brain (ratio)
E(T)	cerebral extraction fraction of butanol integrated to time of decapitation (ratio)
$E_i(T)$	extraction fraction of butanol in organ indi-

cated by subscript o at time of decapitation (ratio)
plasma flow rate per unit weight of brain (ml (100 g) min)
blood flow rate per unit weight of brain (ml (100 g) min)
blood flow rate per unit weight of an organ indicated by subscript "o" (ml (100 g) min)
total plasma flow to organ indicated by subscript o rate of collection of arterial plasma (ml min)
total blood flow to organ indicated by subscript o" rate of collection of arterial blood (ml min)
cardiac output (ml kg min)
hematocrit (ratio)
unidirectional blood brain glucose transfer rate per unit weight of brain (μmol (100 g) min)
rate of monoexponential decay of arterial labeled butanol concentration (s)
half saturation constant of unidirectional blood-brain D-glucose transfer (mM)
brain blood partition coefficient for butanol (ml g)
P mean arterial blood pressure (mmHg)
number of perfused capillaries per unit weight of brain ((100 g))
rate constant of labeled butanol turn-over in brain (s)
arterial oxygen tension (mmHg)
arterial carbon dioxide tension (mmHg)
arterial pH
permeability surface area product of unidirectional blood brain transfer of solutes (ml (100 g) min)
content of labeled D-glucose in organ indicated by subscript o at time of decapitation amount of labeled D-glucose collected in arterial syringe (nCi)
content of labeled butanol in organ indicated by subscript o at time of decapitation amount of labeled D-glucose collected in arterial syringe (nCi)
total amount of labeled butanol given at time t=0 (nCi kg)
time after intravenous bolus injection (s)
time from intravenous bolus injection to peak of arterial labeled butanol concentration curve (s)
mean transit time of plasma in brain ( )
time spent in cerebral capillary (s)
time of decapitation (s)
maximal rate of unidirectional blood-brain glucose transfer per unit weight of brain (μmol (100 g) min)
maximal rate of unidirectional blood-brain glucose transfer in a small section of an average single cerebral capillary (μmol min)
volume of plasma in small section of average single" cerebral capillary (ml)
V volume of capillary plasma per unit weight of brain (ml (100 g))
V volume of plasma per unit weight of brain (ml (100 g))

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ltrastructure of neurosecretosomes sedimented in the nuclear fraction from the posterior pituitary of the rat

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Subcellular fraction of the posterior pituitary of the rat were isolated by differential and density gradient centrifugation and microsamples prepared for electron microscopy by $KMnO_4$ or glutaraldehyde-OsO_4 fixation The nuclear fraction P (1 000 g 7 min), was the main neurosecretosome (NSS) fraction and contained nuclei and mitochondria in addition to NSS (60%). The crude mitochondrial fraction, P (10 000 g 20 min), contained free mitochondria (70%) NSS unidentified membrane particles fat droplet derived from pituicytes, neurosecretory granules (NSG) and synaptosomes, identified by the presence of synaptic membrane thickenings. Fraction P (32 900 g 70 min) contained mainly free NSG ble fraction P (118 000 g 30 min) had high microsome content.

Key word Posterior pituitary subcellular fractionation, neurosecretosomes, synaptosomes, ultrastructure

It is well known that pinched-off nerve endings (synaptosomes) isolated from the brain sediment in differential centrifugation in the mitochondrial fraction and can be purified partly by centrifugation in density gradients, as a result of which they sediment in lighter subfractions than mitochondria (De Robertis & Rodriguez de Lores Arnaiz 1969 Whittaker 1969). The sedimentation characteristics of synaptosomes are so uniform that attempts to separate them into subclasses according to the transmitter or regional specificity have met with only limited success. There are a few exceptions to this uniformity The large mossy fibre synaptosomes from the cerebellum sediment in the nuclear fraction in differential centrifugation (Hajós et al. 1974). Another deviation is seen with synaptosomes from developing brain, which sediment in the mitochondrial fraction but during subfractionation show a wide variation of densities, ranging from the lightest to the usual density (for ref. see Hervonen et al 1974 Kanerva et al 1977).

Several studies have been carried out on the subcellular fractionation of the posterior pituitary mainly using bovine tissue because of the small size of the organ The results obtained in differential and density gradient centrifugation indicate that pinched-off neurosecretory nerve endings (neurosecretosomes NSS) from the posterior pituitary have a sedimentation density higher than that of mitochondria and ordinary synaptosomes, though lower than that of nuclei (La Bella et al 1965 Bindler et al 1967 Lederis & Livingstone 1970 Vilhardt & Baker 1976 Boer et al 1976 Vilhardt et al 1977). By employing a micromethod for the preparation of samples for electron microscopy Cotman & Flansburg 1970) we used glutaraldehyde-osmium tetroxide and $KMnO_4$ fixation to study the fine structure of subcellular fractions from the posterior pituitary of the rat. Our finding was that neurosecretosomes sediment in the nuclear fraction. Non-neurosecretory nerve endings have also been isolated from the posterior pituitary Pre-

liminary findings have been reported (Kanerva et al 1978)

MATERIAL AND METHODS

Virgin female and male Sprague-Dawley rats 2–3 months old were used The subcellular fractionation procedure was that used by Bindler et al (1967) with slight modifications In each experiment which was repeated several times about 30 rats of either sex were decapitated with a guillotine between 9 and 11 a m The posterior pituitary was immediately dissected out under a stereomicroscope (40×) at 4°C and placed in 0.32 M sucrose in an ice bath Pooled tissues were homogenized in 9 vol of 0 3 M sucrose in a Teflon/glass homogenizer with a clearance of 0.25 mm (Kontes) by 10 strokes at 1 000 rpm The cylinder was rinsed 4 times with the same volume of 0 32 M sucrose The homogenate and washings were combined and centrifuged differentially at 1 000×g for 7 min (fraction P) at 10 000×g for ?0 min (fraction P) at 3 900×g for 20 min (fraction P_3) and at 118 000×g for 30 min (fraction P) leaving a particle-free supernatant, S_4. Fraction P was washed once with 1 ml of 0.3 M sucrose and the washing combined with supernatant S_1 In some expts fraction P was suspended in 0 32 M sucrose and layered over a density gradient consisting of 1 ml of 0 1 74 1 45 and 1 16 M sucrose and centrifuged at 55 000×g for 1?0 min Subfractions A B and C sedimenting above 1 16 1 45 and 1 74 M sucrose respectively were collected by vacuum aspiration in each case including half of the clear zone between the layers diluted with water to a sucrose concentration of 0.55 M allowed to stand for ?0 min and centrifuged at 40 000×g for 30 min. Differential and density gradient centrifugations were performed in a Spinco L 50 or L 3-50 Beckman ultracentrifuge at 4°C using an SW 50 1 or SW 39 L rotor all g values were calculated at R_{av} The pellets obtained from differential and density gradient centrifugations were suspended in Krebs bicarbonate solution pH 7 4 (KRB) After 20 min at 0°C aliquots were removed for electron microscopy and occasionally for protein assay (Lowry et al 1951). In some expts. the incubation of the P suspension wa continued at 37°C and 95% O +5% CO After 8 min of prewarming 100 μg/ml 5-hydroxydopamine (H 83/35 Hassle Goteborg) 10^{-4} M pargyline HCl (Eutonyl Abbott Chicago Ill) and 0._ mg/ml ascorbic acid (final concentrations) were added and the incubation continued for 15 or 30 min

Samples for electron microscopy were prepared by the micromethod of Cotman & Flansburg (1970) 100–?00 μl aliquots of the suspension (about 0 1 mg of protein) were transferred to siliconized bottleneck Beem capsules (Ted Pella Los Angeles Cal) in an ice bath and fixed as suspension or pellet for electron microscopy The samples were sedimented by centrifuging them in an SW _5 1 or SW 27 rotor equipped with suitable adapters at 40 000×g for 30 min P_4, however was centrifuged for 90 min The following fixatives were used (all concentrations final) 1) 3% glutaraldehyde in 0.1 M phosphate buffer or 0 05 M cacodylate buffer pH 7 4 a a suspension for 15 min or as a pellet for 1 h 2) 3% KMnO in 0 1 M phosphate buffer pH 7 0 as a suspension for 15 min (Richardson 1966). The samples fixed with glutaraldehy were postfixed in phosphate-buffered 1% OsO at pH for 1– h The specimens were then dehydrated in grad ethanol and embedded in Epon-Araldite. The specime fixed in KMnO were viewed and photographed stained while the sections fixed in glutaraldehyde-O were stained on grids with lead citrate and uranyl aceta A Philips EM 300 electron microscope operated at 60 was used

The estimations of relative particle areas were m according to the principle laid down by Lead (196 grid consisting of 13 parallel lines was placed over electron micrographs ?0 electron micrographs taken without any selection from each fraction at magnification (×8 700) Micrographs were taken fro the various regions of the pellet to ensure that they representative

RESULTS

The nuclear fraction P (1 000 g×7 min Fig contained in order of frequency (relative part area) NSS (about 60%) nuclei (15%) an heterogeneous mixture of free mitochondria (10 15% of fraction P comprised small numbers of droplets from pituicytes free neurosecre granules (NSG) pinched-off nerve terminals i tified by the presence of synaptic membrane th enings (synaptosomes) fragments of blood ves and collagen fibres and unidentified memb fragments

The neurosecretosomes showed the same d structural variations as those seen in tissue sect (for ref see Baumgarten et al 197?). Their con (Fig 1) varied from complete filling with elect dense NSG to a mixture of NSG and small vesic and solitary NSG with abundant small vesicles. NSS generally had a diameter of over 1 μm th both smaller and very large NSS were detected

After glutaraldehyde-osmium tetroxide fixat the NSG were filled with electron-dense mat (see Fig) Sometimes the granules appeared empty because of a probable extraction of mat during the preparation procedure The NSG tions varied in diameter between 100 nm and nm with a mean diameter of about 160 nm (as culated from 100 NSG) The NSG were surrou by a unit membrane which was best visual after KMnO fixation This trilaminar structure identical in the NSG microvesicles (MV) and ronal outer membrane Other organelles in the were the small mitochondria neurotubuli vacu and some multilamellated bodies NSS with de erative features were also fou —



Fig. 1. Nuclear fraction P after 3% KMnO fixation in suspension. General view of the area with large number of well preserved pinched-off neurosecretory endings (neurosecretosomes) containing neurosecretory vesicles (arrow) and microvesicles (arrow head). The cytoplasm has leaked out from some neurosecretosomes on the right (star), giving the neurosecretosome light background. 20 175

After KMnO fixation (Fig. 1) the core of the NSG consisted of a reticular substructure; no dense core was observed. As was the case after glutaraldehyde-osmium tetroxide fixation this reticular substructure was missing in some granules, indicating leakage of the protein-peptide complex during the preparation procedure. The MV were agranular after the use of this fixative.

The crude-mitochondrial P fraction (10 000 g × 20 min) contained (in order of frequency) free mitochondria (70%), NSS (15%), unidentified membrane particles (10%), fat droplets derived from pituicytes, NSG and synaptosomes. The nerve endings most frequently detected in this fraction, too, were those of the neurosecretory type which contained NSG and clear MV 30 to 60 nm in diameter. A small number of nerve endings were detected without NSG but containing synaptic membrane thickenings, i.e. synaptosomes. These were divided into two groups: 1) monoaminergic (MA) characterized by the presence of small dense-cored vesicles after incubation with 5-hydroxydopamine and fixation with $KMnO_4$, and 2) non-monoaminergic (probably cholinergic) characterized by the presence of clear synaptic vesicles (data not shown). After density gradient centrifuga-

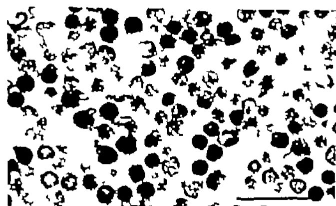


Fig 2 Fraction P Glutaraldehyde-OsO fixation A relatively pure fraction of neurosecretory granules ×30 500

tion of fraction P_2 NSS were found in subfractions B and C sedimenting above 1 45 and 1 74 M sucrose respectively

Fraction P (37 900 g×20 min) contained an abundance of free NSG (Fig 2) small membrane fragments and MV Fraction P (118 000 g×30 min) had a high content of small membrane fragments and MV together with a few NSG

DISCUSSION

La Bella et al (1965) in their first study divided homogenates of the bovine posterior pituitary into 4 fractions sedimenting at 15 000 63 000 255 000 and 1 020 000 g-min As shown by electron microscopy NSS sedimented in the 63 000 g min fraction and NSG in the 255 000 g min fraction In a subsequent study (Bindler et al 1967) these authors divided the homogenates into 6 fractions sedimenting at 7 000 23 300 41 000 164 000 658 000 and 3 550 000 g min here NSS sedimented in the 23 300 g min fraction and NSG in the 658 000 g-min fraction When the NSS fraction was centrifuged in a density gradient consisting of 1 16 1 45 and 1 74 M sucrose NSS were found above all the sucrose concentrations Vilhardt & coworkers (Vilhardt & Baker 1976 Vilhardt et al 1977) also working with the bovine posterior pituitary separated them into fractions sedimenting at 12 000 45 000 75 000 390 000 and 6 000 000 g-min and characterized the fractions by hormonal and enzymatic markers They used electron microscopy to verify that NSS sediment in the 45 000 g-min fraction and after density gradient centrifugation at the region of 1 4–1.5 M sucrose and that NSG sediment in the 390 000 g min fraction Lederis & Livingstone (1970) studied the subfractions of the crude mitochondrial fraction obtained from the posterior pituitary of the rabbit and found that nerve endings have a wide variation of densities ranging from 1 0 to 1 7 M sucrose Rat posterior pituitaries have recently been investigated by Boer et al (1976) who prepared subcellular suspensions by a microdispersion method and fractionated them directly in a Ficoll density gradient NSS sedimented mainly at the 14/22% Ficoll interphase in contrast to brain synaptosomes which sediment at the 7.5/13% Ficoll interphase (Gonatas et al 1971)

It is clear from the studies referred to above that NSS from the posterior pituitary have a sedimentation density higher than that of synaptosomes In none of these studies however were NSS found in the heaviest nuclear fraction a finding in the present study Other completely new findings were non neurosecretory nerve endings identified by synaptic junctions in subcellular fractions from the posterior pituitary These had previously only been found in slices (Baumgarten et al 1972). One possible reason why neurosecretosomes in the present study sedimented in the 7 000 g min fraction might be the difference in species fractions of differential centrifugation from the rat posterior pituitary have not been studied previously Another possible explanation could be the immediate processing after death which resulted in good preservation of the electron-dense contents of NSG as seen in the preparations fixed by glutaraldehyde-osmium tetroxide (Fig 2) A time delay after death might cause loss of some constituents and a decrease in sedimentation density Indeed Boer & Van Rheenen Verberg (1976) have found that after water deprivation and a decrease in vasopressin content there is a change in the sedimentation of NSS from the 14/22% Ficoll interphase to the 10/14% Ficoll interphase

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Muscarinic autoreceptor regulates acetylcholine release in rat hippocampus: in vitro evidence

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Release of ^{3}H-ACh from isolated nerve endings of rat hippocampus was evoked by incubation in Krebs-Ringer buffer containing 25 or 35 mM potassium. The release was Ca^{2+}-dependent and could be inhibited by Mg^{2+} (20 mM). The muscarinic antagonist atropine (10^{-10}–10^{-6} M) enhanced ^{3}H-ACh-release. The muscarinic agonist carbachol (10^{-7}–10^{-4} M) inhibited ^{3}H-ACh release via interaction with muscarinic receptors; this effect could be blocked by atropine (10^{-6} M). The presence of the feed-back regulation of ^{3}H-ACh release in a cell free preparation provides further evidence that the presynaptic regulation is exerted by muscarinic autoreceptors localized on the cholinergic nerve ending itself. The feed-back inhibition of the ^{3}H-ACh release does not require the presence of intact neurons or neuronal loops; tetrodotoxin ($2.5 \cdot 10^{-6}$ M) does not affect the above results.

Key words: Acetylcholine, acetylcholine release, atropine, autoreceptor, presynaptic, synaptosome

The importance of presynaptic receptors in the regulation of norepinephrine release is well documented (cf. Starne 1975, Starke et al. 1977). Involvement of muscarinic acetylcholine receptors in regulation of acetylcholine (ACh) release from cerebral cortex in vivo (Mitchell 1963, Szerb 1964, Jaffer & Szerb 1969) and in vitro (Polak & Meeuws 1966, Molenaar & Polak 1970, Polak 1971, Bourdois et al. 1974, Kato et al. 1975) has been demonstrated more than a decade ago. Muscarinic regulation of ACh release on the periphery has also been shown (Schaumann 1956, Kilbinger & Wagner 1975, Sawynok & Jhamandas 1977). Detailed studies on electrically evoked release of ACh from striatal, hippocampal and cortical slices by Szerb and colleagues showed that acetylcholinesterase inhibitors lower the release of ^{3}H-ACh (Bourdois et al. 1974) whereas muscarinic antagonists restore or enhance ACh release (Szerb & Somogyi 1973). Furthermore, it was shown that septal lesion which destroys cholinergic afferents to the hippocampus leads to decreased release of ^{3}H-ACh from hippocampal slices of lesioned animals (Szerb 1977, Szerb et al. 1977). However, in binding studies with the potent muscarinic antagonist, ^{3}H-quinuclidinylbenzilate (^{3}H-QNB), Yamamura & Snyder (1974) were unable to detect any loss of muscarinic receptor sites in lesioned hippocampus. Thus there is an apparent contradiction between the results indicating the presence of a muscarinic presynaptic receptor that regulates the release of ACh from hippocampus and between the ability of septal lesions to decrease ACh release but not to diminish the number of muscarinic binding sites. Several possible explanations were offered (Szerb 1977) to resolve this contradiction. (*a*) muscarinic regulation of ACh release is accomplished through interneurons, thus the number of receptors is not reduced by the lesion which only destroys the afferents; (*b*) the antagonist, ^{3}H-QNB, has so low affinity for the presynaptic receptor that the concentrations used in the binding studies (1 nM) though sufficient to label the postsynaptic muscarinic receptor (Yamamura & Snyder 1974) did not label the presynaptic receptor. Consequently, loss of the latter upon the lesion escaped detection.

Whether or not the muscarinic receptors which participate in the feed-back regulation of ACh release in the hippocampus are localized on the cholinergic nerve endings cannot be concluded from the studies carried out in tissue slices as the presence of interneurons can not be excluded in

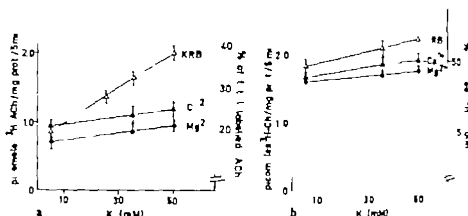


Fig 1a Dependence of H ACh release on K concentration in the medium The osmolarity was held constant by decreasing Na concentration to the same extent as K was increased The KRB was composed as described in Experimental Procedures. −Ca² −▲− did not contain Ca^{2+} and was supplemented with 0 3 mM EGTA. Mg^{2+} −●− contained 20 mM Mg^{2+} instead of 1 mM The data are expressed as means ± S.D. of triplicate determinations. The experiment was carried out 3 times.
Fig 1b Dependence of release of H-choline on K concentration in the medium. Conditions were as in Fig 1*a*

such systems Polak (1971) on the basis of experiments on ACh release from slices in the presence of tetrodotoxin (Molenaar & Polak 1970) suggested that the muscarinic receptors that regulate ACh release are localized on cholinergic nerve terminals In the present study we addressed the question whether in a cell-free system of nerve endings from rat hippocampus ACh release can still be regulated via a muscarinic receptor The studies carried out on dilute suspension of synaptosomes in the presence of tetrodotoxin lend further support to the idea that feed-back regulation of ACh release is exerted by muscarinic autoreceptors localized on the cholinergic nerve ending itself

EXPERIMENTAL PROCEDURES

Preparation of nerve ending

5 male Sprague Dawley rats (150–180 g) were decapitated and the hippocampi dissected and placed in ice-cold sucrose medium 0.32 M Tris/HCl 5 mM pH=7 4 and EDTA 40 µM Preparation of P pellet enriched in nerve endings and mitochondria followed the procedure described by Sgaragli et al 1977 In brief 10% (w/w) homogenates were prepared by homogenization at 395 rpm in a loose fitting glass-Teflon homogenizer After 5 up-down strokes the homogenate was allowed to stand on ice for 5 min before repeating the homogenization The homogenate was diluted to yield a 10% solution and centrifuged at 1000×g for 5 min. The supernatant was further centrifuged at 15000×g for 12 min, and the resulting pellet was rinsed twice The experiments were carried out using the P fraction (Experiments with synaptosomes prepared according to Jones & Matus (1974) gave the same results.

However the yield of synaptosome preparations was l and the obtained nerve endings were often "leaky" 4

Labelling of nerve endings (4 mg/ml) th ^{3}H-cho (total concentration 8 µM H-choline 0.3 µM) as carried out according to Sen et al. (1976) in Krebs-Ringer buf (NaCl 138 mM KCl 5.3 mM $MgCl_2$ 1 mM NaH_2PO_4 mM $NaHCO_3$ 11 mM glucose 10 mM NaOOCCH mM and $CaCl_2$ 1 mM) for 30 min at 37°C under continuo bubbling with 95% O_2–5% CO gas mixture At the end incubation the suspension was centrifuged 1 500 g for min at 4°C and the pellet was washed 3×5 ml cold Kre Ringer s buffer supplemented with physostigmine mM)

Release of H-acetylcholine

The H-choline labelled pellet was resuspended to yiel mg/ml protein concentration in ice-cold Krebs-Ringe buffer with physostigmine (0. mM). The release v studied at 37°C for 5 min in glass centrifuge tubes conta ing the drugs studied and K at 5 5 mM or 35 concentration (Increase in K concentration was co pensated for by reducing the Na concentration) I ncubation was carried out in a final olume of 100 containing 100–150 µg protein. All release expts. carried out in the presence of 3-hemicholinium (HC 10 µM) and tetrodotoxin (TTX) (5 µM). ^{3}H ACh and choline were separated by high voltage (3000 V) pap electrophoresis according to Hildebrand et al (19 using triethylammonium marker as described by Lad & Consolo (1974) After the run the paper strips were into 0 5 cm wide pieces and placed in H_2O (1 ml) ov night before addition of 10 ml scintillation fluid (Lumag Lumac Systems AG Basel) The samples were counted a Beckman scintillation spectrometer Protein as e mated by the method of Lowry et al (1951).

Tritium labelled choline (methyl- H-choline) chlor (31 Ci/mmol) and H-acetylcholine chloride (0 25 mmol) were purchased 1 Ci/

...ble 1. Uptake and release of ³H-choline and the release of ³H ACh from rat hippocampal synaptosomes

...r experimental details, see Experimental Procedures. The amount of protein present during labelling with ³H-choline ...85 µg. Data from two other experiments agree within 15 %

	Picomoles/mg protein	Picomoles
³H-Choline present in the uptake medium (5 ml)		1 550
³H-Choline uptake into the synaptosomes	1_5	231
³H-ACh in the synaptosomes	5.5	102
³H-Choline in the synaptosomes	3.8	70
³H-Choline-derivatives (phosphatidyl)choline etc. that did not migrate with authentic ³H-choline)	3.2	59
...al ³H ACh release (5 mM K)	0.9	17
...oked ³H-ACh release (35 mM K)	1.5	28
...al ³H-choline release (5 mM K)	1.9	35
...oked ³H-choline release (35 mM K)	2.1	39

...ham, England Tetrodotoxin was bought from ...ringer AG, Stockholm, and oxotremorine from EGA ... Steinheim, West Germany; all other reagents were ...ducts of the Sigma Chemical Co., St. Louis, Miss., ... All solutions were made up daily

RESULTS

...polarizing concentrations of K evoked re-...ase of ³H-ACh in the presence of extracellular ...²⁺ (Fig. 1) Potassium (50 mM) doubled the ...ase observed at K (5 mM). The latter release ...ll be termed basal or resting release while the ...e observed in the presence of higher than 5 mM ... concentrations will be referred to as evoked ...ease. Omission of Ca² concomitant with appli-...tion of EGTA (0.3 mM) or application of high ...g²⁺ concentrations (20 mM) abolished most of the high K evoked release without effecting the basal release (Fig. 1*a*). The resting release or leakage during 5 min at 37°C accounted for ~70 % of the total labelled H ACh (cf. Table I). Though 50 mM K produced larger release of H ACh and H choline than did 35 mM K for studies on the effect of muscarinic ligands on the release this concentration (50 mM) was not suitable. At K concentrations above 35 mM the responsiveness of the system towards atropine and carbachol fell rapidly. Thus most of the experiments were carried out at 5, 25 or 35 mM K concentrations. (The release of ³H-choline was much less sensitive to depolarizing concentrations of K or changes in Ca² concentrations (Fig. 1*b*).)

Electrophoretic separation of the released material was obligatory since ³H ACh represented a

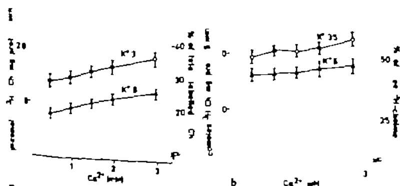


Fig. 2. Dependence of H ACh release on extracellular Ca²⁺ concentrations in the presence of K (5 mM) –△– K (35 mM) ○—○ The data are expressed as means ± S.D. of triplicate determinations. The experiments were carried out 3 times.
Fig. 2*b*. Dependence of ³H-choline release on extracellular Ca² concentrations. Conditions were as in Fig. 2*a*.

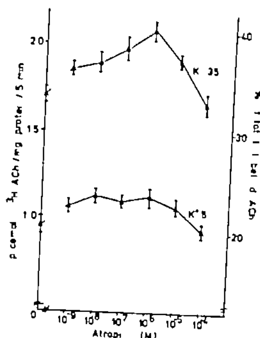


Fig 3 The effect of various atropine concentrations on the basal (K 5 mM) Δ—Δ and evoked (K 35 mM) ▲—▲ release of H ACh The data represent means ± S D of quadruplicate determination The increase in release of H ACh in the presence of atropine concentrations was significantly ($p<0.1$ $p<0.05$) different from the control value at the same K concentration

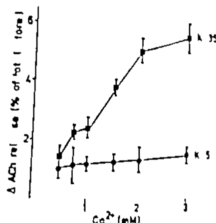


Fig 4 Ca² -dependence of the efficacy of atropine enhancing H ACh release Acetylcholine release is the difference observed at 5 mM K in the presence and absence of atropine (1 μM) at 5 mM K ●—● and the difference in H ACh release in the presence and absence of atropine (1 μM) at 35 mM K ■—■

different portion of H-labelled material released in 5 mM K vs in 35 mM K containing medium

Both basal and evoked release of H ACh were dependent on the extracellular Ca² concentration (Fig 2A) The release of H-choline showed a much smaller dependency on the extracellular Ca² concentration (Fig 2B)

Atropine in a dose-dependent manner increased release of ³H ACh at 5 and 35 mM K concentrations (Fig 3) Enhancement of the evoked release (35 mM K) was much greater than that of the basal release

To test the assumption that this finding reflects that the more released ACh is present the more apparent the effect of atropine is experiments were carried out at different Ca² concentrations

Fig 4 shows that the efficacy of atropine defined as release in the presence minus release in the absence of atropine (10⁻⁶ M) is greatly increased with increasing Ca² concentrations in the case of evoked release The efficacy of atropine in enhancing basal ³H ACh release increased much less with increasing Ca² concentrations

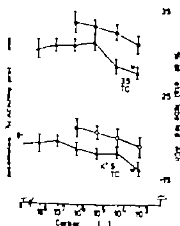
Atropine also enhanced the release of H-choline with 10–25% at both 5 and 35 mM K concentrations (data not shown)

The release of H ACh and the effects of atropine on ³H ACh release were not influenced by addition of tetrodotoxin (2.5 μM) or by addition or omission of 3-hemicholinium (10 μM)

To examine the effects of a muscarinic agonist on H ACh release the experiments were carried out in the presence of carbachol d Tubocurarine (dTC) (10 ⁵ M) a nicotinic antagonist was present to block nicotinic actions of carbachol d Tubocurarine itself had no effect on the basal or evoked release (Table 2) Carbachol (10 ³ M) in the presence of dTC (10 ⁵ M) inhibited both basal and evoked release of H ACh (Table 1)

The weak inhibitory effects of carbachol on H ACh release could be relieved by 10 ⁶ M atropine (Fig 5) At 1 mM carbachol concentration μM atropine blocked the inhibition on ³H ACh release

Several other drugs were tested in this release system The muscarinic agonists oxotremorine (10 M) methacholine (10 M) were very weak inhibitors of evoked release of H ACh in the presence of 0.2 mM physostigmine The inhibitory effect of muscarinic agonists could be observed easier in the absence of any cholinesterase inhibitor (physostigmine or Sarin) but the data showed much greater variability The cholinesterase inhibitors physostigmine (0.2 mM) and Sarin (0.1 mM) had inhibitory effects on both basal and evoked release (Table 2)



i 3 Influence of cholinergic drugs: carbachol atropine l and d-tubocurarine (dTC) on H-ACh release. d bocurarine 10⁻⁵ M as present in all expts. depicted on figure Atropine, ben added, was present at 10 M centration p<0.1 significantly different from control no carbachol present at the same K concentrations)

SCUSSION

gulation of acetylcholine release from hippocam- s by muscarinic receptors has been well docu- nted (cf. Szerb 1977) Whether this feed-back bition of the release involved neuronal loops or receptors located on the cholinergic nerve end- itself was, however controversial.

It seemed thus desirable to examine the presence he muscarinic feed-back regulation in a cell-free pension of nerveendings from the hippocampus ch systems do not contain intact neurons or ronal loops and drugs can reach the nerve ings freely (Mulder et al. 1975).

Release of the neurotransmitter was evoked by polarizing concentrations of K (25 or 35 mM) her than by electrical field-stimulation K acts ectly on the nerve endings and permits simul- eous use of tetrodotoxin (2.5 μM) to ensure that pulse conduction in neuronal loops is not res- nsible for the observed feed back regulation

As shown in Figs. 1 and 2, depolarizing concen- tions of K (25 or 35 mM) release ³H ACh and I-choline in a Ca²⁺-dependent manner from the rve endings Thus the system fulfils requirements f a physiological release system (Electrical sti- mulation (0 6–10 Hz, 20 ms duration for 5 min) or ouabaine (10⁻ 10⁻⁴ M) can also be used to re ase ³H-ACh in this system) As shown in Figs. 1 and 2, both H ACh and ³H-choline were released at 5 and 35 mM K concentrations, however re- lease of H choline showed a lower degree of de- pendency on the K concentrations and on the Ca² concentrations The fraction of total labelled ³H ACh that was released during a 5 min incubation with elevated K concentration is 20–40 % This figure is rather high when compared to fractional release from intact nerves or organs however it should be noted that (*a*) we do not know how much of the total ACh is released since we follow a rapid- ly turning over labelled pool of H ACh and (*b*) that it is assumed that the nerve endings do not regenerate normal membrane potential after K depolarization. The fractional release of ACh from *Torpedo ocellata* (Michaelson et al 1979) and from hippocampal synaptosomes (Nemeth & Cooper 1979) was of similar magnitude (basal release ~20 %)

Atropine enhanced the evoked (K 35 mM) re- lease of H ACh This effect on ³H ACh release clearly indicates the presence of muscarinic recep- tors on nerve endings from hippocampus. The half maximal effect was attained between 10⁻⁹–10⁻⁸ M concentrations which are well comparable to the value of binding constants of H-atropine to homo- genates of rat brain (Alberts & Bartfai 1976) Szerb (1977) has found that the half maximal atropine effect in enhancing ³H ACh release from slices was

Table 2. *Th effect of variou drug on H-ACh release from synaptosom from rat hippocampu*

Drug	Percentage of basal release K =5 mM	K =35 mM
No drug present	100±17	157±10
Atropine (1 μM)	106±14	182±17*
Physostigmine (200 μM)	71±11	114±15
Sarin* (100 μM)	66±15	117±11
Tetrodotoxin (2.5 μM)	104±15	16.±21
d Tubocurarine (10 μM)	105± 9	179±19
Carbachol (1 mM)	91±17	124±13*
Carbachol (1 mM) + d-tubocurarine (10 μM)	67±11	125±20*
Carbachol (1 mM) + d-tubocurarine (10 μM) + atropine (10 μM)	114±19	165±21

From single experiment carried out in triplicates.
p<0 1 is significantly different from no drug pre- sent" at the same K concentration. All data represent the mean ± S.D. for 6 independent experimental points 100% basal release of H-ACh corresponds to 0 92±0 14 picomol ³H-ACh/mg protein ×5 min.

about 17 nM The efficacy of atropine increased with the increased concentrations of ^{3}H ACh that were present Thus the efficacy of atropine was higher at higher Ca^{2+} concentration (Fig. 4)

A draw-back of the system used is that release of ^{3}H ACh was measured rather than release of endogenous ACh Since it is known that ACh occurs in several pools in the nerveendings (Simon et al 1976) and in the superior cervical ganglion (Birks & MacIntosh 1961) it is not possible to draw any conclusion concerning the total release of ACh on the basis of data on release of H ACh Nevertheless the existence if not the extent of any regulatory mechanisms governing release of ACh can be established by the system used Our results yield evidence in support of the hypothesis by Polak (1971) that feed-back regulation of acetylcholine release from hippocampus is exerted via muscarinic presynaptic receptors which are localized on the cholinergic nerve ending and therefore act as autoreceptors

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brain extracellular space during spreading depression and ischemia

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HANSEN A. J & OLSEN C E., Brain extracellular space during spreading depression and ischemia. Acta Physiol Scand 1980 108 355–365 Received 23 June 1979 ISSN 0001-6772. Institute of Medical Physiology Department A University of Copenhagen and Chemistry Department, Royal Veterinary and Agricultural University Copenhagen, Denmark.

The change of xtracellular space volume of rat brain cortex during ischemia and cortical spreading depression CSD (Leão 1944) was evaluated by a new method The cortical surface was irrigated with isotonic CSF containing the extracellular markers 50 mM cholin or 50 mM trimethyltris(hydroxymethyl)methyl ammonium ion (N TRIS), and their extracellular concentrations were monitored by ion-selective microelectrodes. When steady-state for the concentration of these markers was attained, CSD evoked re versible increase of the concentration of the markers, indicating shrinkage of the inter stitial volume of distribution. During ischemia an initial slow rate of concentration increase was observed, followed few minutes later by rapid increase concomitant with the sharp rise in xtracellular potassium concentration During CSD and ischemia the maximal increases of choline and N-TRIS concentration reflected a shrinkage of the extracellular space amounting to about 50% of the initial olume

Key ord Brain extracellular space ion-selectiv microelectrodes, choline cortical spreading depression ischemia

he ionic composition of brain extracellular space s subject to significant changes during ischemia and rtical spreading depression (CSD). The con-entrations of sodium, chloride and calcium de ease (Dora & Zeuthen 1976 Hansen 1979 Kraig & Nicholson 1978 Nicholson & Kraig 1975) while he potassium concentration undergoes rapid eleva-ion (Dora & Zeuthen 1976, Hansen 1977 1979 Nicholson & Kraig 1975 Vyskočil et al 1972) Absolute movements of ions between the extra- and intracellular spaces cannot be calculated without knowledge of the variation of the volume of the extracellular space

The distribution volume of extracellular markers, s inulin, is believed to be the most valid x pression of the size of the extracellular space (Katz man & Pappius 1973) but the method requires a long equilibration time and subsequent tissue analysis

The method here presented is based on con-tinuous determination of the extracellular con-centration of extracellular markers and is therefore able to detect rapid changes of brain extracellular space volume

MATERIALS AND METHODS

In brief, the method was applied in the following way After removal of the dura, the exposed parietal cortex was irrigated with isotonic mock CSF containing 50 mM choline chloride or 50 mM trimethyltris(hydroxymethyl)-methyl ammonium chloride (N TRISCl). The extra-cellular concentration of these substances in the brain cortex was measured by means of microelectrodes during the course of CSD or brain ischemia. Since both sub-stances have low cellular permeability rapid changes of the concentration reflect changes of the size of the extra-cellular space.

Theory

Upon application to the surface of the brain, choline or N-TRIS diffuses into the cerebral cortex through the

... ational pentobarbital as given when necessary A ... ethylene tube as placed through tracheostomy ... all polyethylene tubes ere inserted into the femoral ... artery and vein for recording of blood pressure and for ... respectively The body temperature was kept at ... °C by servo-controlled heating system. The animal ... breathing spontaneously throughout the experiment. ... the head as secured in headholder the cal... was exposed and hole (about 6 mm in diameter) ... drilled the left parietal bone A smaller hole was made ... the left frontal bone about 5 mm in front of the large ... A perspex ring, 7 mm in inner diameter and 5 mm ... was glued to the bone of the large hole using East... 910 adhesive The dura as removed, care being ... not to damage the underlying pia. The EEG was ... recorded with platinum pins inserted beneath the perio... of the cranty of the larger hole. I three animals, ... electrocorticogram as recorded from small silver ... placed lightly on the parietal corte with no inter... with the local blood supply The animal were ... grounded th glass tube filled with 150 mM ... solidified in agar placed in neck muscle or in the ... tissue on the right side of the head.

Microelectrode and solutions

Double-barrelled potassium-selective microelectrodes ... tip diameter of about 1 μm were constructed and ... as described by Hansen (1977) The electrodes were ... filled with potassium ion exchanger (Corning 477317) ... exchanger has previously been show to be sensitive ... quaternary ammonium ions (Neher & Lux 1973 ... & Simon 1976, Scholer & Simon 1977)

The parietal cortex was irrigated with mock CSF con... in mmol l^{-1} CholineCl (ChCl) 50 NaCl 75 KCl ... $CaCl_2$ 1.3, $MgCl_2$ 1.1 $NaHCO_3$ 25 NaH_2PO_4 0.5 ... and Glucose 3.4. ChCl replaced 90 mmol 1 ... of NaCl The pH as adjusted to 7.4 and the osmolarity ... 295 mosmol l^{-1} In three animals, ChCl was replaced ... by 90 mmol l^{-1} of trimethyltris(hydroxymethyl)methyl ammonium chloride (N-TRISCl), which is ordinary Tri... where the hydrogen atoms in the amino-group have been substituted by methyl groups N-TRIS possesses more hydrophilic groups (CH_2OH), presumably making the molecule less permeable in the cell membrane The electrodes were calibrated with 38°C artificial CSF con... ChCl in the following concentrations (mmol l^{-1}): 1.5, 10, 20, 40, 60 and with solutions with 10 and 100 mmol l KCl For calibration of N-TRISCl the con... in the artificial CSF was 50, 25 12.5 6.25 ... 3.13 (mmol l^{-1}). The increase of ChCl, N-TRISCl and ... KCl as balanced by corresponding decrease of NaCl ... to preserve isotonicity

The selectivity constants of choline and N-TRIS with respect to potassium ere determined from an extended Nikolsky equation

$$E = E_0 \frac{RT}{z_i F} \ln (C_i \;\; K_{ij} \;\; [K]^{z_i/z_j}) \qquad (4)$$

where E the electrode potential E_0 constant reference potential, C_i the concentration of choline or N-TRIS K_{ij} the selectivity constant, z_i and z_j the valences of choline or N-TRIS and potassium, respectively and RT/F the Nernst factor

Trimethyltris(hydroxymethyl)methyl ammonium chloride (N TRISCl)

To tirred mixture of tris(hydroxymethyl)methyl amine (0.244 g) sodium hydrogen carbonate (1.0 g) and dimethyl sulfoxide (3 ml) was added methyl iodide (0.7 ml) in the course of 30 min. Stirring at room temperature was continued for 22 h After dilution with methanol, the solid material was filtered off Water was added to the filtrate and the solution passed through an ion e change column (Amberlit IR 120 70 1 cm, H -form) After thorough washing with water the product was eluted with N HCl After evaporation to drynes on rotary evaporator the material was extracted with hot ethanol. Filtering, evaporation and recrystallization from ethanol gave 0.326 g (81%) of almost pure compound (NMR). The compound was recrystallized once more using ethanol–2-propanol as solvent, before being used for the physiological experiments.

M p 241°C (uncorrected gas evolution) H NMR spectra were recorded in D_2O on JEOL C-60 HL instrument, using ....3,3 -tetradeuteno-3-(trimethylsilyl)-propionate as internal standard NMR data, δ 3.35 (9H singlet), 4.06 (6H slightly broadened singlet) 4.7 (singlet) (Found. C 41.84 H 9.04 N 6.96 Calc. for $C_7H_{18}NO_3Cl$ C 42.11 H 9.09 N 7.01)

Experimental procedure

In order to increase brain extracellular concentration of choline or N TRIS, the parietal cortex was xposed to warm (38°C) CSF containing 90 mmol l^{-1} of the particular test substance The perspex ring around the large hole functioned as reservoir and the fluid in it was renewed at least every 15 min

In three experiments, the ion-selective electrode was positioned at selected depths in the corte before super fusion with the choline or N-TRIS-CSF in order to follow the initial rise of concentration. In other animals, laminar determinations of the concentration were made at different times after onset of irrigation. The CSF was soaked up and the electrode quickly lowered into the cortex by means of mechanically driven micromanipulator The level at which the electrode made contact with the cortex was considered zero depth. CSF was then added to the reservoir The electrode was lowered into the cortex and readings of the concentration taken every 100 μm down to depth of 1 000 μm.

In order to assess the distribution of choline or N-TRIS between intra- and extracellular space extracellular concentration profiles were obtained after 0.5 2, and 4 h exposure, during which period CSD or ischemia were not elicited Then the surface was quickly flushed with normal CSF the rats decapitated, and 0.75 mm thick slice of cortex cut off with strong knif (Franck et al. 1968) A sample of the cortical slice from the irrigated part was homogenized by ultrasound with water added The choline and N-TRIS concentration in the sample was determined with microelectrode calibrated with similarly homogenized control brain samples with known amounts of choline or N-TRIS added The concentrations in brain water were calculated assuming water content in brain cortex of 80.5% From the measured concentration profiles, mean concentration of choline or N TRIS in the

extracellular space Assuming diffusion in one dimension and ignoring carrier mediated transport the change of the extracellular concentration can be treated mathematically by a model concept as shown below

Consider a layer of cortical tissue situated at depth x oriented parallel to the brain surface (i e perpendicular to the direction of the diffusional transport) and having an area of 1 cm^2 and a thickness of dx In this tissue element of volume dx cm^3 and with an extracellular concentration of tracer substance $C_r = C_r(x\ t)$ the change in content of tracer substance within time dt in its extracellular space can be expressed by a term for diffusive net entrance and a term for loss by penetration through cell membranes (i e brain cell membranes and capillary walls) Assuming the intracellular and blood concentration to be zero the equation becomes

$$dC_r\ dx\ f_e = [J(x) - J(x+dx)]\ dt - \bar{P}_{app}\ S\ dx\ C_r\ dt \qquad (1)$$

where J is diffusive flux (per cm^2 of brain area) f_e is fractional volume of extracellular fluid in cortical tissue and S is membrane surface area (per cm^3) of cortical tissue $\bar{P}_{app}$ is an apparent weighted mean membrane permeability the magnitude of which depends partly on the permeabilities of the membranes through which penetration occurs and partly on electrical potentials existing across the membranes It is therefore a weighted mean of the apparent permeabilities of the various categories of membranes each of which again is the actual permeability multiplied by a factor determined by the transmembranal potential Assuming that penetration oc curred exclusively into cells with identical intracellular potential (V) and that the penetration of the monovalent cations in question occurs only by electrodiffusion the relation between $\bar{P}_{app}$ and the actual permeability P can be *calculated from the Goldmann flux equation*

$$\bar{P}_{app} = P\ \frac{VF}{RT}\ \frac{\exp-\{(VF)/(RT)\}}{1-\exp-\{(VF)/(RT)\}}$$

where F R, and T have their usual meaning For V = −60 mV the result is $\bar{P}_{app}$ = P 2.5 i e a uniformly existing transmembranal *potential of this magnitude would cause* an apparent increase in the permeability by a factor of 2.5 Rearranging eq (1)

$$\frac{\partial C_r}{\partial t} = -\frac{\partial J/f_e}{\partial x} - \frac{\bar{P}_{app}\ S}{f_e}\ C_r \qquad (2)$$

and inserting,

$$J(x) = -D\ f_e \frac{\partial C_r}{\partial x} \text{ and } \frac{\bar{P}_{app}\ S}{f_e} = \frac{\bar{P}_{app}\ S\ C_r}{f_e\ C_r} = \frac{1}{\tau}$$

where $1/\tau$ is a rate constant for penetration through the cell membranes (i e. the fraction of the marker contained in the extracellular volume which leaves it by penetration per unit time) the *governing* equation is obtained

$$\frac{\partial C_r}{\partial t} = D\frac{\partial^2 C_r}{\partial x^2} - \frac{C_r}{\tau} \qquad (3)$$

which describes diffusion with superimposed penetra into an infinitely large sink.

Eq (3) assumes unidirectional flux into cells a capillaries The treatment thus disregards a possible crease of the net flux with time caused by an eff from the intracellular volume or a fraction of it, a concentration of marker is built up in these volumes the influx Since choline and N TRIS are positiv charged their tendency to leave cells is low compa to the influx as long as the membrane potential is the order of −40–80 mV and intracellular concentra below extracellular concentration. Since choline a N TRIS are both quarternary ammonium compounds w all three N-linked hydrogens substituted by met groups they will be positively charged at all preval pH values

The increase of the extracellular concentration in h cortex following the onset of irrigation can be solved the boundary conditions

$$C_r(0\ t) = C_0$$
$$C_r(x\ 0) = 0$$

by Laplace transformation. The result is,

$$C_r(x\ t) = \tfrac{1}{2}C_0\left\{\exp\left(-\frac{x}{L}\right)\operatorname{erfc}\left(\frac{x}{\sqrt{2\ Dt}} - \frac{t}{\tau}\right) + \exp\left(\frac{x}{L}\right)\operatorname{erfc}\left(\frac{x}{\sqrt{2\ Dt}} + \frac{t}{\tau}\right)\right\}$$

where $L = \sqrt{D\tau}$ is the characteristic diffusion length e extracellular space When the extracellular conten tracer substance is constant with time i e

$\frac{\partial C_r}{\partial t} = 0$ eq (3) *reduces to* $D\frac{\partial^2 C_r}{\partial x^2} = \frac{C_r}{\tau}$

the only physically meaningful solution of which is,

$$C_r(x\ \infty) = C_r(0\ \infty)\exp\left(-\frac{x}{L}\right)$$

Thus the concentration in the cortex in this situ decreases exponentially with distance and therefore yi a straight line in a semi-logarithmic plot.

Determination of D and

The time course of the concentration was measure different depths in the cortex following irrigation of surface From the concentration versus time curve tained at various depths *D and were found numeri* by insertion of different sets of D τ values into eq (4

In the steady state concentrations in the cortex plotted versus depth (up to 1 mm) on a logarithmic s and $L = \sqrt{D\tau}$ determined as the abscissa value responding to a concentration of 1/e of the concentr at the surface

Preparation of nimals

Male Wistar rats weighing 400–500 g were used They were initially anaesthetized with lobarbital (50 mg/kg)

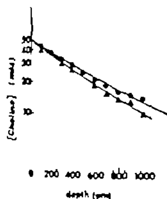


Fig. 3. Extracellular choline concentration (logarithmic scale) in rat brain cortex following irrigation of brain surface with isotonic mock CSF containing 50 mM of cholineCl for 2 h (▲) and 4 h (●). The symbols represent mean values from 3 rats.

Extracellular concentration of choline and N-TRIS versus time

Irrigation of the parietal cortex with artificial CSF containing 50 mmol l⁻¹ choline or 50 mmol l⁻¹ N-TRIS neither interfered with the electrical activity of the cortex nor affected the appearance of the cortex.

Fig. shows the rise of extracellular choline concentration at two depths in the cortex following onset of irrigation. The dotted lines represent the experimental readings, the unbroken lines the best fits using eq. (4) with the following parameters. At 350 μm, $D=1\times10^{-6}$ cm² s and τ=25 min. At 800 μm, $D=2\times10^{-6}$ cm² s and τ=.5 min. D for choline in saline is 14×10^{-6} cm² s⁻¹ (Fröhner 1910). A similar experiment with the electrode placed at a depth of 400 μm, using N TRIS gave $D=1.5\times10^{-6}$ cm² s and τ=60 min.

Extracellular concentration of choline and N-TRIS versus depth

Fig. 3 shows the extracellular choline concentration, measured every 100 μm during electrode penetration into the parietal cortex, after 2 and 4 h superfusion with 50 mM choline-CSF. CSD or ischemia was not elicited prior to the determination of the depth profile. The values were plotted semilogarithmically and it is likely that the profiles at 2 and 4 h are sufficiently close to indicate that the extracellular content of tracer substance was constant after 4 h. It is, however, not a steady state since in that situation the intracellular concentration of tracer substance must exceed the extracellular concentration by a factor of ten (see below) assuming an intracellular potential of −60 mV. From the 4 h values in Fig. 3, L, the characteristic length (eq. (5)) was determined to be 740 μm. As $L=\sqrt{D\tau}$ and assuming =.5 min this gives $D=4\times10^{-6}$ s.

Intracellula concentration of chol ne and N TRIS

Fig. 4 shows the calculated mean intracellular concentration of choline and N TRIS in the outermost 0.75 mm of cortex following arious times of irrigation. For both compounds, there is a linear concentration rise with time. After 4 h the mean extracellular concentration of choline in two rats was 31 mM and 29 mM while the concentration of choline in the water phase of the homogenized brain was 33 mM and 29 mM respectively: thus at this time nearly equal mean concentrations should be present in the intra- and extracellular compartment. In the rat irrigated with N TRIS-CSF for 4 h, the mean concentrations in extracellular water and in brain water were found to be 31 mM and 4 mM respectively.

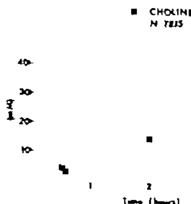


Fig. 4. Mean intracellular concentration of choline or trimethyltris(hydroxymethyl)methyl ammonium ion (N TRIS) in the outermost 0.75 mm of rat parietal cortex, irrigated with isotonic mock CSF containing 50 mM of cholineCl (■) or 50 mM or N TRISCl (▲) for 0.5, 2, and 4 h. The intracellular concentration was determined from the total cortical concentration and the extracellular concentration, assuming an extracellular space of 20%.

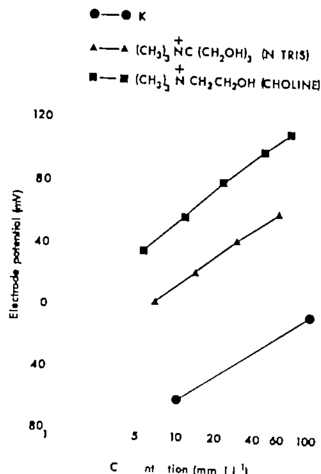


Fig 1 Calibration curves for an ion selective microelectrode with K -ion exchanger (Corning 417377) against KCl (●—●) trimethyltris(hydroxymethyl)methyl ammoniumCl (▲–▲) and cholineCl (■–■) NaC was added to the potassium solutions to preserve isosmolality The two latter compounds were dissolved in mock CSF and replaced equimolar amounts of NaCl

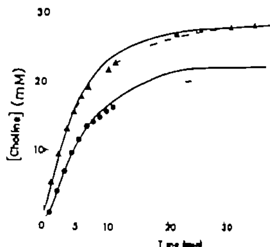


Fig 2 The rise of choline concentration in the ex cellular space in rat brain cortex following irrigation parietal cortical surface with mock CSF in which 50 NaCl was replaced by 50 mM cholineCl The cho concentration was measured by an ion-selective mic electrode at a depth of 700 μm (▲) and 400 μm (●) T unbroken lines represent best fits to the experimen data using a particular combination of diffusion co ficient for choline and time constant τ (see Methods)

extracellular space of the outermost 0 75 mm of cortex was determined From this concentration and a sumption that f_e amounts to 20% or brain cortex volume a mean concentration in the corresponding intracellular water was calculated

With the electrode positioned at depths ranging from 200–700 μm CSD's were elicited from the cortex beneath the small hole in the frontal bone by a drop of saturated KCl-solution or by quickly pinning a needle into the cortex CSD was monitored by the transient change of the brain extracellular potential (V -potential) so-called DC-potential (Leão 1947)

Cerebral ischemia was induced either reversibly by inflation of a pneumatic cuff placed around the neck (Siemkowicz & Hansen 1978) or irreversibly by ar resting the heart by infusion of 0.3 ml saturated $MgCl_2$-solution through the venous catheter In order to a oid artefacts from movements of the brain during early ischemia, the electrode was in some cases removed and quickly repositioned after the cuff had been inflated

Results are expressed as mean ±S E

RESULTS

Electrode sensitivity to choline and N TRIS

The mixed solution technique yielded l $K_{choline\ K}$ = −2.2 indicating the electrode to b about 150 times more sensitive to choline than K in agreement with the finding of Scholer Simon (1972) Similarly log K TRIS K was Since the extracellular K concentration rises to above 60 mM during CSD and ischemia (Hanse 1977 1979 Vyskočil et al 1972) the cholin induced potentials were measured in solutions of mM ChCl in 60 mM KCl or 60 mM NaCl Like wise N TRIS-induced potentials were measured i solutions of 25 mM N TRIS with either 60 m KCl or 60 mM NaCl No differences were detecte among the K and Na -containing solutions

Fig 1 shows calibration curves obtained wi pure solutions of ChCl N TRISCl and KCl For choline there was an increase in potential ap proximately 66 mV for a 10-fold change in con centration above 20 mmol l and 75 mV below 20 mmol l For N TRIS there was a potential change of approximately 65 mV for a 10-fold change in the whole range The reason for this "super Nernstian slope is presently unknown

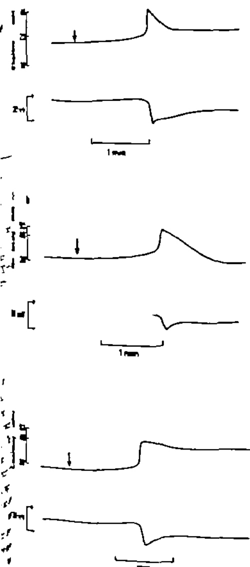


Fig. 7. Changes of extracellular parietal cortical concentrations of the two extracellular space markers during ischemia. Arrows indicate heart arrest produced by rapid injection of 0.3 ml saturated $MgCl_2$-solution. The cortex was irrigated with isotonic mock CSF containing either 40 mM cholineCl (*a*) or 50 mM trimethyltris-(hydroxymethyl)methyl ammonium ion (N-TRIS) (*b*), and the concentration of these markers measured approximately 0.5 mm below cortical surface by double barrelled ion-selective microelectrodes. Lower curve of each figure represents change of brain extracellular potential. The duration of irrigation was 1 h in ( ) and (*b*), and 4 h in (*c*). Note the decline of marker concentration following the steep increase after irrigation of 1 h, while after 4 h the concentration remained at a high level.

shot should decrease with duration of exposure to marker substances. Since the brain cells are depolarized during CSD (Higashida et al. 1974, Sugaya et al. 1975) the driving force for transport into the cells is diminished when the concentrations in the intra- and extracellular spaces have become equal (at about 4 h for choline, cf. above). When CSD was elicited after longer irrigation periods, the early decline disappeared (Fig. 5*b*) and the undershot was diminished. Fig. 6 shows that the undershot—relative to the pre-existing concentration—after the first CSD was related to the length of exposure to the marker substance. When ~3 CSD's were elicited repetitively the pre-CSD level of tracer substance dropped for every CSD and the undershot was progressively diminished. After these CSD's, the concentration of tracer substance returned slowly to a level similar to that before the elicitation of the CSD's due to diffusion from the cortical surface. Thus when several CSD's were elicited, irrespective of duration of irrigation, the undershot of the last CSD was similar to the undershot of the first CSD elicited after 4 h of irrigation. This observation indicates that CSD promoted uptake of tracer substance into cells.

We can make a rough estimate of the extent to which cellular uptake influences the maximal level attained during the steep tracer increase by assuming the time constant for cellular uptake to be similar to the time constant for the concentration decline observed immediately following the end of the steep increase. The amount of choline M taken up by the cells of 1 cm^3 of cortex equals P_{max} S C_e t = f_e l/τ C_e t. In Fig. 5 the choline concentration doubled in s during the steep increase, and $t_{\frac{1}{2}}$ for the early decline was 10 s. During the 2 s, the extracellular choline concentration and the fractional volume of extracellular fluid changes from [$C_e(1)$ $f_e(1)$] to [$C_e(2)$, $f_e(2)$]. Assuming that the increase of intracellular choline concentration is negligible and that f_e and C_e change linearly $M = \frac{1}{2}(f_e(1)+f_e(2))$ 0.0693 s $\frac{1}{2}(C_e(1)+C_e(2))$ s. The amount of choline taken up by the cells can also be expressed as $M = f_e(1)\ C_e(1) - f_e(2)\ C_e(2)$. Since $C_e(1) = 22$ mM and $C_e(2) = 42$ mM (Fig. 5*a*) the fractional change of extracellular space volume calculated as $C_e(1)/C_e(2)$ is $f_e(2)/f_e(1) = 0.52$, whereas when cellular uptake is taken into account, the calculation yields $f_e(2)/f_e(1) = 0.45$. Thus, the fractional change in extracellular volume, calculated from the fractional change in the choline concentra-

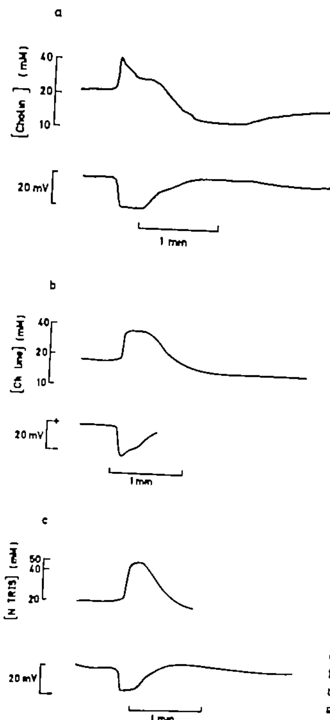


Fig 5 Change of parietal cortical extracellular concentration of the two extracellular space markers during the course of spreading depression The cortex was irrigated with isotonic mock CSF containing 50 mM of cholineCl for 1.3 h (*a*) or 4 h (*b*) Note greater undershot at 1.3 h. In (*c*) the cortex was irrigated with mock CSF containing 50 mM of trimethyltris(hydroxymethyl)-methyl ammonium ion (N TRIS) for 1 6 h Concentration of the markers was measured approximately 0.5 mm below cortical surface Lower curve of each figure represents change of brain extracellular potential Spreading depression was elicited from frontal cortex either by placing a drop of saturated KCl-solution or by a stab with a small needle

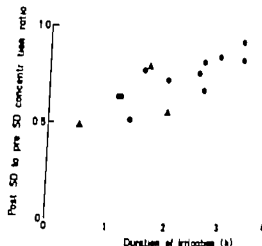


Fig 6 Relationship between duration of irrigation parietal cortex with isotonic mock CSF containing 50 mM of cholineCl (●) or 50 mM of trimethyltris(hydroxymethyl)methyl ammonium chloride (N TRISCl) (▲) and maximal undershot of the extracellular concentration of these substances after the first CSD expressed as fraction of pre-CSD level Spreading depression was elicited as described in Fig 5

Changes during cortical spreading depression (CSD)

The changes of concentration of choline (1? rats) and N TRIS (3 rats) during CSD were similar and will be described together Fig *5a* shows the change of extracellular choline concentration during CSD after 1.3 h of irrigation The change of V potential resembled the change observed in rats with normal composition of the brain extracellular space Coinciding with the rapid negative deflection of the V potential a steep elevation of the choline concentration occurred in ? s Immediately followed by a decline which after about 1 min undershot the pre-CSD level Fig *5b* shows CSD in a rat after choline exposure for 4 h Note that it shows a similarly steep increase of the choline concentration as in Fig *5a* but the increase is followed by a plateau phase and there is less undershot Fig *5c* shows the change of the N TRIS concentration during a CSD after 1 6 h of irrigation A similar concentration change as that of Fig *5b* was found

The early decline of choline concentration immediately after the steep increase (Fig *5a*) and the undershot after CSD could reflect expansion of the extracellular space or removal of marker substance from it If cellular uptake was involved the decline should occur at a slower rate and the under

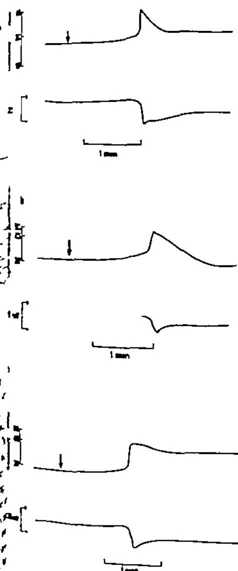


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tion may be 15% too low The $t_{\frac{1}{2}}$ for the early decline observed in other CSD s was larger than 10 s and accordingly no correction was performed

The change of the choline electrode potential during the steep increase of the first spreading depression in 11 rats averaged 19±0 9 mV while the change for all later CSD s averaged ?2±0 7 mV (n=23) corresponding to an increase from 17 mM to 33 mM and from 17 mM to 35 mM respectively (i e increases by 94% and 105%) In all rats the duration of the steep increase was 1–2 s In ten rats where more than one CSD (?–7) was elicited the undershot after the last CSD was 5±1 1 mV corresponding to a choline concentration of 85% of the pre-CSD level The five CSD s in three N TRIS-treated rats caused an average electrode potential change of 1±3 mV during the steep increase corresponding to a concentration increase from 23 mM to 47 mM (i e increases by 104%) The undershot after the second CSD in two of the rats was 6 mV (81% of pre-CSD level)

Changes during ischemia

No apparent differences were noted between the results obtained with choline and those obtained with N TRIS Fig 7 shows the change of the extracellular marker concentration and V potential during brain ischemia The change of the V potential is similar to the change observed in experiments where the extracellular space contained no foreign ions (Hansen 1977 Vyskočil et al 1972) Immediately following the heart arrest induced by $MgCl_2$-injection the choline concentration slowly began to increase At the steep negative deflection of the V potential (anoxic depolarization) a 2 fold elevation of choline concentration occurred rapidly This pattern was observed in all ischemic episodes but the concentration attained after the steep increase depended on the duration of exposure to marker substance If the surface of the brain was irrigated for approximately 1 h before ischemia, the concentration declined after the steep increase (Fig 7*a b*) After an exposure time of 4 h however the level remained almost stable after the steep increase (Fig 7*c*)

The fall of marker concentration after the anoxic depolarization could be due to expansion of the extracellular space or more likely to cellular uptake since the concentration fall was more pronounced after short time exposure i e when the intracellular marker concentration was low (Fig The duration of the steep increase was 1 3 s (a $t_{\frac{1}{2}}$ of 1–3 s) while the $t_{\frac{1}{2}}$ for the initial post p cellular uptake was above 15 s in all isch episodes Because of the short duration of the st increase the cellular uptake of choline or N-TI may be considered to have had a negligible effect their extracellular concentrations during the st increase (cf section on Changes during cor spreading depression (CSD))

The increase of choline potential during the p depolarization phase i e before the steep incre was 6±1 0 mV corresponding to an increase fi 16 mM to ?0 mM and during the steep incre 17±1 1 mV (from ?0 mM to 35 mM) (i e an crease to a level 25% and 119% above the ischemic level respectively) In the three N TI treated rats the values were 6±1 1 mV (from 2 to ?7 mM) for the predepolarization phase 17±1 ? mV (from 27 mM to 45 mM) for the st increase (23 and 105% above the pre-isch level respectively)

In a few instances the change of the ch concentration was studied during and after isch induced by strangulation with a pneumatic cuf a duration of 5–10 min The increase of ch concentration during ischemia was reversible fastest rate of decline being observed during malization of the V potential This finding ag with observations of the normalization of e cellular potassium concentration after a perio ischemia (Hansen et al 1979) In association brain swelling noted in the microscope the p ischemic choline concentration undershot control level presumably by forcing the tip of electrode into deeper structures with lower ch concentrations No brain movements were served during the course of irreversible ischemi nor during CSD

DISCUSSION

The present investigation aimed to measure th change of extracellular space of brain cortex durin CSD and ischemia Under the assumption that th concentration change of choline and N TRIS re flects volume changes in the extracellular wate phase the size of the extracellular space decrease to 50% during CSD Under the same assumption the space shrank dur[illegible] to 80% of th

...stant value during the predepolarization phase and ...% during the anoxic depolarization.

Choline and N TRIS as extracellular space markers

Choline was chosen as a marker for the extracellular space for three reasons. First, it is generally believed that cell membranes are not or only very slowly penetrated by choline The compound is often used in studies in which physiologically important ions such as potassium or sodium must be replaced by an inert cation. Secondly choline permeability of the nerve cell membrane remains low in situations in which the ionic permeability is increased, e.g. during the action potential change (Hille 1971 Hodgkin & Katz, 1948) Thirdly choline can be accurately measured by means of ordinary potassium-sensitive microelectrodes with insignificant interference from potassium ($k_{...}$ = 0.006) N-TRIS can be measured with the same technique and was selected because it is likely to be less permeable in cell membranes (cf below)

It is unlikely that the change of marker concentration observed during CSD and ischemia is not influenced by transport of markers to or away from the extracellular space, since even in the normal state considerable cellular uptake was observed The cellular uptake of choline in our experiment occurs almost exclusively by electrodiffusion since facilitated diffusion would only increase the intracellular concentration from approx. 30 μM (Schwab 1976) to 3 mM in 4 h, assuming a maximal transport capacity of 10 nmol g min (Carroll & Goldberg 1975). But the substances are still useful extracellular markers, if it can be shown that the time constant, for transport from the extracellular space including transport through capillary walls (Cornford et al 1978) is much higher than for the change of the extracellular space volume In the normal state, for transport from the extracellular space as above 25 min However the for transport from the extracellular space during CSD and ischemia is unknown but can be inferred from the change of marker concentration occurring immediately after the steep increase By comparing for the change in marker concentration during the steep increase with for the marker concentration changes immediately after the end of the steep increase, it was shown that loss of marker substances from the extracellular phase affected the calculation of the volume changes during the steep increase only negligibly The presence of this significant albeit slow change of marker concentration following the steep increase indicates an increase of brain cell permeability to the present tracer substances It invalidates any interpretation of choline and N TRIS concentrations in terms of extracellular volume for the entire period of time following and possibly prior to the steep increase and it further suggests that the steep increase of marker concentration itself could not be the result of cellular release.

Extracellular changes during CSD and ischemia

Maintenance of cellular volume is commonly described by the double Donnan system (Macknight & Leaf 1977) according to which the effect of the osmotic pressure of the intracellular impermeable anions is counteracted by extrusion of sodium. When this mechanism fails, cells swell because water follows sodium and chloride into the cells (Kow & Van Harreveld 1972, Macknight & Leaf 1977 Van Harreveld 1966)

The finding of large decreases of extracellular concentration of sodium and chloride during CSD and ischemia (anoxic depolarization) (Hansen 1979 Kraig & Nicholson 1978 Nicholson & Kraig 1975) is in agreement with the result of the present study During the pre-depolarization phase in ischemia only small changes of extracellular sodium- and chloride concentration take place but cellular swelling could be due to accumulation of anaerobic metabolites intracellularly Several other works within recent years support the occurrence of extracellular volume changes during the conditions studied above

Electrical impedance of brain tissue is considered an index of the volume of the extracellular space (Van Harreveld 1972) During CSD a transient 2 fold increase of impedance was found (Hoffman et al 1973) and in ischemia, a slight increase was found during the pre-depolarization phase followed by a rapid rise during which the impedance almost doubled (Van Harreveld & Ochs 1956)

Brain extracellular space has been studied electronmicroscopically using freeze-substitution in order to preserve the water distribution between the intra- and extracellular spaces (Van Harreveld 1972) During CSD and ischemia, the volume of the

extracellular space decreased and cells swelled (Van Harreveld & Malhotra 1965 Van Harreveld & Khattab 1967) A volume increase of cerebral corti cal cells during anoxia measured by light scatter ing has also been reported (Lipton 1973) Altogether the results referred to above are in good agreement with those obtained by the present tech nique showing shrinkage of the extracellular space to half its initial value during CSD and ischemia

The authors wish to thank Ms M Anker Sorensen for expert technical assistance O Christensen Ph D for the mathematical treatment Professor C Crone A Gjedde M D H Lund Andersen M D and Professor P Olesen-Larsen for valuable advice and support and Ms B Ree for expert typing of the manuscript

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Renal blood flow distribution during E. coli endotoxin shock in dog

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KIRKEBO A. & TYSSEBOTN I. Renal blood flow distribution during E. coli endotoxin shock in dog. Acta Physiol Scand 1980. 108: 367–372. Received 3 July 1979. ISSN 0001-6772. Institute of Physiology, University of Bergen, Norway.

The effect of endotoxin on renal blood flow distribution was studied in anaesthetized dogs. Renal blood flow was measured as hydrogen clearance by platinum electrodes placed in outer and in inner halves of cortex and by electromagnetic flow meter. Intravenous injection of E. coli endotoxin 3–5 mg/kg b. wt. promptly reduced arterial blood pressure (AP) and renal blood flow. After transient increase for 45 min AP and renal blood flow declined to about 50% of the control 2½–3 h after injection. The reduction in outer cortical blood flow (OCF) was not significantly different from the reduction in inner cortical blood flow (ICF). The hematocrit (Hct) increased from 40.1±3.8% to 54.6±8% but mean renal vascular resistance did not change. Total plasma protein concentration was not significantly elevated. A marked local flow variability was observed in some periods during the phase of shock with declining AP and total renal blood flow at high Hct. Thus renal blood flow showed phasic changes but the OCF/ICF ratio was not changed during endotoxin shock. Local blood flow instability was observed periodically at high Hct.

Key words: Renal blood flow distribution, hydrogen clearance, endotoxin

...ly ischemic necrosis of the outer renal cortex has been produced by injection of staphylococcus ... in rabbits by several investigators (Neisser & ... 1901, Navasquez 1938, Trueta et al. 1947). ... and colleagues postulated that renal blood ... was diverted from "cortical" glomeruli toward ... medullary glomeruli and thereby caused ... re... in urinary output. A selective reduction of ... flow in outer cortex was confirmed by ... et al. (1977) by measuring [85]Kr concentra... in frozen kidney pieces from dogs in endotoxic ... and by Neiberger & Passmore (1978) record... washout by external detection. Similar ... were obtained also by Cronenwett & Lin... (1978) counting microspheres in septic dog ... They held that washout of the medullary ... concentration gradient by increased jux... medullary blood flow could explain the inappro... diuresis observed in their experiments.

However, such redistribution has never been ... in any model by use of H_2 washout technique ... Hollenberg et al. (1970) observed a homogene... ous reduction of cortical perfusion in septic patients by external counting of [85]Kr.

During dehydration shock in dog, Kirkebo & Tyssebotn (1977) observed patchy intermittent ischemia equally distributed in both cortical layers. The flow instability was most frequently encountered at medium reduction of arterial pressure and renal blood flow, and always at high Hct. Septic shock is more often characterized by higher cardiac output and renal blood than other types of shock (Schoemaker 1977), although conflicting results have been reported for the dog (Hinshaw et al. 1961, 1968). Hct is also known to increase rapidly.

Therefore, the aim of the present investigation was to measure renal blood flow distribution by H_2 washout technique during endotoxin shock and to search for the appearance of patchy intermittent changes in cortical blood flow at high Hct. The random and relatively rare occurrence of sudden changes in local flow in the present work, made it difficult to investigate possible mechanisms explaining this phenomenon.

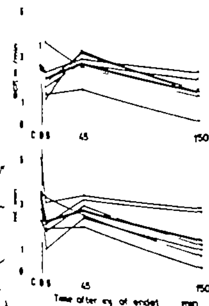


Fig 2 Outer cortical blood flow (OCF) and inner cortical blood flow (ICF) in control and after injection of E. coli endotoxin 6 dogs. Symbols represent mean local flow from 3 electrodes. Average values given by solid lines.

Hct was 40 1±3 8%. The renal arterial blood flow (RAF) averaged 3 63±0 77 ml/min g in accordance with local outer cortical blood flow (OCF) of 3.59±... ml/min g and inner cortical blood flow (ICF) of 3.0±... ml/min g. The H_2 washout curves in control periods were all monoexponential down to at least 10% of initial saturation. TPP was ...±0 17 g/100 ml.

Effects of endotoxin

Endotoxin administration caused variable hemodynamic responses, but a general pattern may be outlined.

Immediately after the injection of endotoxin AP and RAF dropped to an average of 56% and 4 % of the control whereas renal vascular resistance (RR) increased by 35% (Fig 1). OCF and ICF were reduced to 57% (2.03±0 90 ml/min g) and to 67% (2.30±0.84 ml/min g) of control about 5 min. after administration (Fig.).

The initial phase lasted a few minutes. AP and RAF thereafter increased rapidly for the next 5–10 min, then more slowly. Both parameters reached maxima of about 85% of control 45 min after starting endotoxin injection. In 5 out of 6 dogs RAF transiently increased to or even exceded the control level (Fig 1). At 45 min average OCF and ICF were increased to 76% (? 74±0 60 ml/min g) and 84% (2.90±0.50 ml/min g) of the control. Hct was raised to 50% but RR had returned to the control values after a transient rise (Fig. 1).

From about 45 min after injection AP and RAF fell gradually so that 3 h after start of endotoxin injection average AP and RAF both were close to 50% of control. Although a great scatter in RR was found average RR remained at control level whereas mean Hct was increased to 54 6±8%. TTP was not significantly different from control. 2½ h after injection of endotoxin OCF was reduced to 44% (1.56±0 73 ml/min g) and ICF to 50% (1 71±1.04 ml/min g). Except for the first 5 min after injection of endotoxin when the OCF/ICF ratio was significantly reduced ($P<0.01$) the OCF/ICF ratio was not significantly different from control throughout the rest of the experiment.

Generally local blood flow at single electrode sites in each dog was well correlated. However in certain periods between 50 and 120 min after endotoxin injection, a pronounced blood flow heterogeneity occurred in all animals detected as uncorrelated flow variations between single elec-

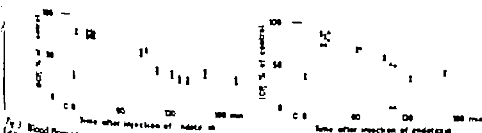


Fig 3 Blood flow at single electrode sites in outer cortex (OCF) and in inner cortex (ICF) in control and after injection of E. coli endotoxin in one dog, showing local flow instability in periods during the shock with declining AP and total renal blood flow.

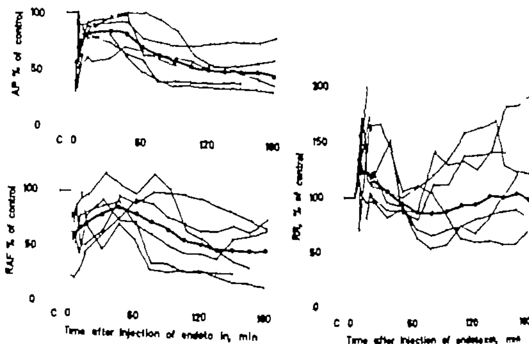


Fig 1 Renal arterial blood flow (RAF) mean arterial blood pressure (AP) and renal vascular resistance (RR) in co (C) and after injection of E *coli endotoxin* in 6 dogs Average values given by solid lines

METHODS

The experiments were undertaken on mongrel dogs of both sexes weighing 21–40 kg mean b wt 26 kg. The dogs had free access to water but food was withheld during the last 16 h before surgery Anesthesia was induced by Nembutal i v 25 mg/kg b wt. and adequate anesthetic level maintained by additional doses The dogs breathed spontaneously through an endotracheal tube Polyethylene catheters were placed in a saphenous vein and artery for infusion and blood pressure recording respectively

The left kidney was retroperitoneally exposed through a flank incision and gently dissected free A polyvinyl catheter was sewn into the renal artery with the catheter tip directed upstream An electromagnetic flow probe (Nycotron) of suitable dimension was placed on the renal artery for continuous determination of total renal blood flow Three L-shaped platinum electrodes with sensitive tapered tips of 0 05–0 15 mm diameter were placed in the outer half and 3 in the inner half of the cortex The shafts of the electrodes were fixed to the renal capsule with 2 sutures and the electrodes carefully covered with perirenal fat. The wound was closed with towel clips.

The H_2 concentration in the tissue around the electrode tips was determined polarographically at a polarization potential of +0.2 V vs an Ag/AgCl electrode placed subcutaneously on the hip (Aukland et al 1973) The electrode current was recorded on a 6-channel recorder (Rikadenki Kogyo Co Model B-64) H_2 saturated saline at 37°C was injected into the renal artery until a stable current was obtained and then abruptly stopped The washout curve was recorded and replotted on a semilogarithmic paper The local blood flow was calculated from the formula $f = -\lambda \ln 0.5/T_{\frac{1}{2}}$ ml/min g where $T_{\frac{1}{2}}$ is half life of H_2-concentration in the tissue around the electrode tips The tissue/blood partition coefficient (λ) assumed to be 1 00 (Aukland et al 1964).

At the end of each experiment the kidney was exc drained for 3 min and weighed This weight was used calculation of total renal blood flow per unit weight the flow meter reading of total renal blood flow

Each electrode was excised and the electrode sampl sites carefully examined for hematoma, and the position the cortex was noted

The E coli endotoxin (Bacto-Lipopolysaccharide coli 0111 B4 Difco Lab) was given i v in a total amo of 3–5 mg/kg b wt. first as a bolus injection of abo mg/kg b wt. and the rest of the dose within 30 min. T experiments were continued for 3 h after start endotox injection

Blood samples were taken approximately each half h to determine Hct and total plasma protein concentrat (TPP) using the amido black method (Munk Plum et 1955)

Statistical significance was evaluated by Student's t-t for paired data.

RESULTS

Control measurements

The control measurements were made 1 h aft completed surgery The average arterial bloc pressure (AP) was 124±S D 7 4 mmHg and mea

shows that compartment analysis of composite washout curves externally detected disagree with total flow measurements and give unrealistic zonal tissue volumes. From tissue sampling technique and interpolation between timed ^{85}Kr concentrations in different dogs Passmore et al (1977) reported that blood flow in outer cortex was reduced more than in inner cortex and outer medulla 30 min after injection of E. coli endotoxin. Their experimental data, however showed great scatter and especially their control washout curves from each of the 3 zones were still multiexponential. The average slope of the regression line used for flow calculation is then strongly depending on the duration of recording. On the other hand, outer cortical washout rate estimated from the initial component would be unreasonably high (ca. 1 min^{-1}) compared to total renal blood flow measurements. The later slow components might indicate large intrarenal low flow regions, but this is not supported by measurements with H_2 electrodes (Tyssebotn & Kirkebø 1979). An alternative and more likely explanation could be recirculation of ^{85}Kr as suggested by Aukland (1980).

Cronenwett & Lindenauer (1978) slowly infused live Pseudomonas aeruginosa for 90 min in awake dogs. Only a small reduction in AP and a small rise in Hct were observed. Most dogs showed increased total renal blood flow and a redistribution of 15 µm microspheres from outer cortex towards inner cortex, as usually has been found with microspheres during renal vasodilation (Stein et al 1973). Contrary to this, we have recently demonstrated that 15 µm microspheres were redistributed in cortex during acetylcholine infusion whereas the distribution of 10.5 µ spheres was not changed as found by H_2 or antipyrine clearance methods (Tyssebotn & Kirkebø 1979, Clausen et al. 1979).

Local blood flow heterogeneity

In the present experiments a marked variability in local cortical blood flow was found in all dogs in periods during development of the septic shock.

It is noteworthy that the present as well as previous findings of intermittent flow changes (Kirkebø & Tyssebotn 1977) were detected only at high Hct values, whereas such changes in local flow were not observed during hemorrhagic hypotension with a low Hct. Normal dogs with a normal blood volume compensated for the rise in Hct and viscosity by a reduction of peripheral resistance (McDonald 1974, Seligman et al 1945). When blood viscosity was raised by exchange of packed red cells for whole blood during hemorrhagic shock, then blood flow was further reduced (Seligman et al 1945). The rise in blood viscosity with Hct is likely to be more prominent at states of low flow as during shock, because blood viscosity increases at low rates of sheer (Usami et al. 1969). Glomerular filtration at high Hct could further rise Hct and viscosity in postglomerular vessels more than at normal Hct. Enhanced aggregation of red cells may also occur at high Hct and during hypoperfusion and has been held responsible for the frequent observations of nonuniform circulation in parallel microvessels during various disease states (Schmid-Schönbein 1976). At the higher than normal yield stress seen during polycythemia (Replogle & Merril 1970) reflow after local occlusion may demand a higher pressure head. Rheological factors may thus sustain hypoperfusion but are less likely to initiate arrest of flow.

Delaunay et al (1949) found that endotoxin produced strong waves of contraction along small arteries in guinea pig mesentery and rabbit ear followed by dilatation, indicating that endotoxin may have effect on vascular reactivity. In the present work, patchy intermittent blood flow occurred mainly at moderately lowered AP and renal blood flow. Increasing concentrations of vasoactive agents are measured in such phases of endotoxin shock (Spink et al 1966) possibly also causing labile vasoactivity. On the other hand, RR was maintained due to increased viscosity and not to reduced vessel diameters. High concentrations of vasoactive agents were found also during hemorrhagic hypotension, but patchy intermittent flow changes were never seen (Aukland et al 1973, Aukland 1976). These observations suggest that rheological factors and labile vasoactivity together induce the sudden shifts in local flow.

In conclusion, E. coli endotoxin decreased local outer and inner cortical blood flow proportionally. A pronounced local flow heterogeneity was observed in phases of shock when Hct was markedly increased.

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trode sites randomly distributed in cortex Local blood flow measurements from one experiment are presented in Fig 3 In 3 dogs sudden sharp changes in washout rate were observed at single electrode sites indicating intermittent and patchy cortical flow distribution without measureable changes in total renal blood flow The local blood flow could remain at the new value from a few seconds up to 1 h before suddenly returning to or approaching the previous value

Three years ago a corresponding observation was made (unpublished) on one dog regarded as unsuitable for planned experiments due to migrating pale depressed patches of 0.5–2 cm² seen on the renal surface The sedimentation rate was increased and the blood culture later verified a *Staphylococcus aureus* sepsis The dog had a normal blood flow (3 2 ml/min g) but during control measurements sudden abrupt bends were recorded on single washout curves The occurrence of intermittent patchy flow changes persisted after α receptor blocking by phentolamine

DISCUSSION

The average values for AP and renal blood flow in control period were within normal ranges although slightly higher local blood flow has been obtained previously by electrodes with thin tip diameter (0 05–0 15 mm) as used in the present work (Tyssebotn & Kirkebo 1979)

The administration of endotoxin caused a sharp transitory drop in AP and renal blood flow as observed also by Dedichen & Schenk (1967) and Hinshaw et al (1968) followed by a shortlasting fairly complete recovery of both AP and RAF After the immediate transitory rise average RR fell rapidly to control within 30 min and remained low or at control level for the rest of the experiment Because Hct rose markedly in the same period and accordingly also blood viscosity (Usami et al 1969) a vasodilation of the renal vessels actually occurred Since plasma protein concentration did not increase significantly the rise of Hct probably was due to leakage of plasma proteins and fluid from the capillaries Severe sepsis in man often exhibits a hyperdynamic state characterized by a low total peripheral resistance and a high cardiac index (Shoemaker 1971 Wilson et al 1965) and renal plasma flow (Lucas et al 1973) On the contrary it has been held that in the dog a fall in cardiac output and a rise in total peripheral resistance occur [illegible] response to endotoxin and that dog experiments therefore represent poor models of the septic patients (Watsbren 1964 Wilson et al 1965 Lucas et al 1973) The septic shock of diverse origin in already severely ill patients may certainly develop differently from acute endotoxin shock in healthy dogs but the differences between species [illegible] have been exaggerated Thus Gombo et al (19[illegible] demonstrated that intravenous administration [illegible] pyrogen in man produced an initial renal vasoconstriction and a subsequent renal vasodilation as seen in our dog While Hinshaw et al in 1[illegible] reported that lethal doses of endotoxin resulted [illegible] renal vasoconstriction they later found that [illegible] was unchanged (Hinshaw et al 1968) Increased renal blood flow was found in dogs receiving [illegible] lethal doses of endotoxin or E coli cells (Dale et al 1976) Hermreck & Thal (1969) likewise measured a 60% increase of renal blood flow in dogs with septic hind limbs Dedichen and Schenk (19[illegible] however found a gradual rise in RR after lethal doses of endotoxin but concluded that the moderate increase was due mainly to decreased vascular filling pressure and increased viscosity rather than to active vasoconstriction Taken together [illegible] is indicated that the procedure for inducing the endotoxin shock may influence the magnitude and duration of vascular responses but that the change of RR in dog is often moderate As RR remained [illegible] control level while Hct rose in the present experiments a fall in renal hindrance was evident

Cortical blood flow distribution

Local cortical blood showed the same phasic responses to endotoxin as RAF The reduction [illegible] OCF was not significantly greater than the reduction in ICF except in the unstable phase 5 min after endotoxin injection

By external counting of ⁸⁵Kr Neiberger [illegible] Passmore (1978) found that 30 min following endotoxin injection component I and II were [illegible] but 2 h later both components were present. Using the same technique with ¹³³Xe Cortez et al (197[illegible] did not find any redistribution of intrarenal blood flow in two septic patients Hollenberg et al (19[illegible] found that the first rapid component disappeared [illegible] patients with renal failure of diverse etiology including nephrotoxin sepsis and shock but [illegible] suggested a persistent homogeneous reduction [illegible] cortical perfusion However Aukland (1976) h[illegible]

Sodium nitroprusside induced cGMP accumulation in isolated frog skin epithelium Effect on cAMP hydroosmotic and natriferic response to antidiuretic hormone

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The cGMP content of isolated frog skin epithelia has been measured and the basal level was found to be 14.3±1.7 fmol/mg dry weight. 0.1 mM sodium nitroprusside induced 10-fold increase in the cGMP level within 5 min after which it rose more slowly The maximum increase in cGMP level was obtained with 1 mM sodium nitroprusside giving 20–30-fold increase 1 mM sodium nitroprusside per se had no effect on osmotic water flow or active sodium transport. On the other hand the osmotic water flow response to arginine vasotocin was somewhat enhanced in skins which had been pretreated with 1 mM sodium nitroprusside; thus the water flow responses to 1 and 31 ng/ml arginine vasotocin were on the average 31 and 14% higher in skins exposed to sodium nitroprusside than in control skins. Sodium nitroprusside had no effect on the increment in sodium transport rate elicited by arginine vasotocin Sodium nitroprusside alone increased the cAMP level slightly: the enhanced cAMP level reached after 30 min incubation with 40 ng/ml arginine vasotocin, was 20% higher in the presence of 1 mM sodium nitroprusside. In conclusion: cGMP has no effect on osmotic water flow nor on active sodium transport and is not involved in the regulation of sodium transport by antidiuretic hormone However cGMP (or sodium nitroprusside) has moderate effect on the hormone-stimulated osmotic water flow

It is well established that ADH increases active sodium transport and water permeability in various epithelia It is also generally accepted that at least some of the effects of ADH are mediated by cAMP (see for example Strewler & Orloff 1977) Whether and how the other common cyclic nucleotide cGMP is involved in salt and water regulation seems less clarified Orloff & Handler (1967) have suggested cAMP and cGMP as mediators of the hydroosmotic and natriferic response to ADH respectively) Bourguignet et al (1969) have found that cGMP in contrast to cAMP has no effect on OWF but like cAMP stimulated SCC This finding has not been confirmed by Bentley (1970) who found no effect of cGMP on OWF or SCC, nor by Sugita et al (1973), who tested several different preparations of cGMP in different Ringers solutions. However Sugita et al. (1973) have found another effect, namely that cGMP enhanced the SCC response to ADH in 2 out of 3 series Piccoli & Parisi (1973) have investigated the combined effect of cGMP and ADH on OWF and found that cGMP increases the basal level a little but strongly inhibits the response to ADH cAMP and to a combination of theophylline and cAMP

Abbreviations used in this paper ADH antidiuretic hormone AVT arginine vasotocin cAMP: adenosine 3',5'cyclic monophosphate cGMP: guanosine 3',5'-cyclic monophosphate OWF osmotic water flow SCC short circuit current, measure of active sodium transport, SNP sodium nitroprusside

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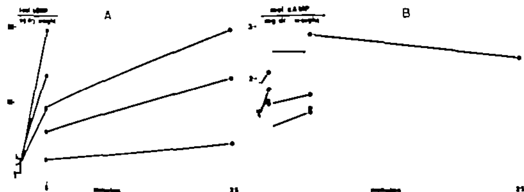


Fig. 2. The effects of varying periods of incubation with 10^{-4} M SNP on concentrations of cGMP (a) and cAMP (b). To compensate for the rather biological variation (especially evident in this figure (a) at 5 and 25 min) the expts were performed as interval studies on symmetrical pieces. 3 series of expts were made by incubating symmetrical pieces for the following periods: 0 and 1 min, 1 and 5 min, 5 and 25 min. The results obtained with symmetrical pieces are connected by lines.

and the dry weight determined. The supernatant was extracted 4 times by 7 volumes watersaturated diethylether and lyophilized. The lyophilizate was dissolved in 500 μl water, placed on a minicolumn (Evergreen Scientific) with 1 g dry Al_2O_3 and eluted with 10 ml water. The eluate was lyophilized, dissolved in buffer and cGMP measured in an aliquot by radioimmunoassay of the acetyl-derivative (Frandsen & Krishna 1976). In an other aliquot cAMP was measured by the method of Gehlar et al. (1977). The purification on Al_2O_3 removes substances which interfere with the cAMP assay (Johnsen & Nielsen 1978). The overall recoveries were typically 55% for cGMP and 75% for cAMP.

The specificity of the cGMP antiserum was tested against 3'-GMP, 5'-GMP, 5'-GDP, 5'-GTP, 2',3'-cGMP, 2',3'-cAMP, 3',5'-cAMP and 5' ATP (GTP was obtained from Sigma, the other nucleotides from Boehringer). Only 3'-GMP, 5'-GDP and 3',5'-cAMP interfered detectably in the assay. If the nucleotides are not lost in the standard extraction and purification procedure the lowest detectable cellular concentrations would be approximately 10^{-4}, 10^{-4} and 10^{-} M respectively. But of these 3 nucleotides only cAMP is eluted from Al_2O_3 (White 1974), therefore only cAMP could interfere in the assay. The actual cAMP concentrations in the epithelium were less than 10^{-6} M (Fig. 2b, Fig. 3b and Table 3. 1 mg dry weight correspond to 2.3 μl cell water), consequently much less than the 10^{-4} M necessary to give interference in the cGMP assay. Thus

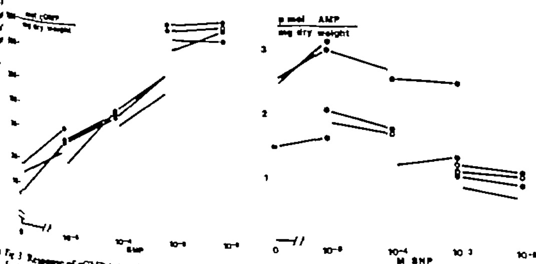


Fig. 3. Response of cGMP ( ) and cAMP (b) to 5 min incubations with different concentrations of SNP. To compensate for the biological variations the expts. were performed on symmetrical pieces, measurements of which are connected by lines. Note the logarithmic scale on the ordinate of

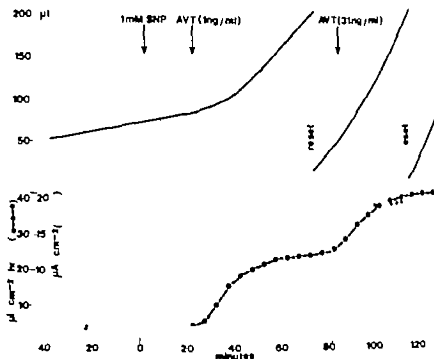


Fig 1 Simultaneous measurements of waterflow and short circuit current using the apparatus described in the text for experimental details see Table .. The upper curve shows the change with time of the volume of the closed half chamber (a 250 µl syringe was used recorder speed was 2 mm/min) The bottom curve shows the first derivative of the upper curve taken each 5 min (0–0–0) calculated as µl h cm Also shown is the short circuit current measured simultaneously ( ) *Reset* the syringe was moved back to the start position and the volume was adjusted by means of a reservoir syringe

Thus it seems possible that cGMP is able to modulate the response to hormonal stimulation of SCC and OWF The authors mentioned above have used exogenous cGMP to increase the intracellular level a procedure which leaves one without any idea of what the intracellular level actually is In the present paper we have used SNP which is known to stimulate cGMP accumulation in a number of tissues (Böhme et al 1978)

The purpose of this work has been to characterize the SNP induced cGMP accumulation in isolated frog skin epithelium and to investigate the relationship to the basal and the ADH stimulated SCC and OWF

METHODS AND MATERIALS

Measurements of cGMP and cAMP content

The experiments were performed on female frogs (Rana temporaria) The frogs were kept partially immersed in tap water at 4°C The skins were divided into 4 pieces two and two symmetrical. The epithelia were isolated by incubation of the skin-pieces with collagenase in test tubes (polyethylene 100×15 mm) containing 5 ml of modified Ringer s solution (Na 115 K 5 Ca 1 Mg 1 C 118 CO 2.5 PO 1 glucose 5 mM pH=7.8) E posure of both in- and outside of the skin to collagenase had no effect other than a minor decrease in responsiveness to stimulation with AVT reported earlier (Johnsen Nielsen 1978) After 1 1.5 h of incubation with collagenase each skin-piece was placed on a glass plate and the intact epithelium was scraped off by means of a microscope slide The epithelium was transferred to fresh medium in a new test tube After 1 2 h equilibration the experiment was carried out under the desired conditions During all incubations the test tubes were placed in a test tube rotator

After the incubation the epithelium was clamped between two brass blocks precooled in liquid N_2. The frozen epithelium was homogenized with 500 µl frozen 6% trichloric acid (TCA) by a steel ball in a vibrating steel capsule (Mikro-dismembrator B Braun Apparatebau) The TCA contained H-cGMP and C-cAMP as recovery markers The steel capsule with content was precooled in liquid N and the temperature ne er exceeded 100°C during homogenization After the homogenization, the capsule was recooled in liquid N and the powder transferred to a preweighed pyrex glass (75×10 mm), where it was stored in liquid N until further processing When desired the homogenate was allowed to thaw in an icebath and centrifuged for 10 min at 10000 g in the same tube The precipitate was dri- ... and 100 mmHg

[illegible] Responses to AVT after 20 min preincubation with 1 mM SNP

[illegible] the application, symmetrical skin halves were preincubated for 20 min in the presence and absence of 1 mM SNP [illegible] AVT (1 ng/ml) was added to both skin halves. The stimulation of OWF and SCC was read 60 min later (values [illegible] % increase = 100 (a–b)/b where b = level before stimulation and a = level 60 min after stimulation) and the [illegible] between the two halves calculated. Then a maximal stimulating dose of AVT (31 ng/ml) was added to both halves [illegible] the total stimulation of OWF and SCC read another 60 min later. The calculations were performed as mentioned [illegible]. Values are mean ± S.E. With a t-test it was tested whether the ratios (+SNP/−SNP) were different from 1.00

AVT dose	Parameter	Unstimulated level	% increase after AVT +SNP	−SNP	+SNP/−SNP	n
1 ng/ml	OWF*	6.1±0.5 µl H_2O/(h cm²)	254±66	229±71	1.31±0.14 $0.05<P<0.1$	8
	SCC	13.9±2.7 µA/cm²	99±34	97±3	1.00±0.04	
31 ng/ml	OWF	6.3±0.6 µl H_2O/(h cm²)	485±114	423±97	1.14±0.03 $0.005<P<0.01$	6
	SCC	14.9±3.6 µA/cm²	154±55	195±82	0.87±0.08 $0.1<P<0.[illegible]$	

* The percentage effect of SNP on the AVT induced OWF was smaller in the skins where AVT induced the larger [illegible] in OWF. This explains the discrepancy between the ratio of the means (254/229 = 1.11) and the mean of the ratios [illegible] the individual experiments (1.31).

[illegible] After a lag period about 1 min the cGMP [illegible] rose approximately 10 times within the next 4 [illegible] and more slowly the following 20 min. 0.1 mM [illegible] induced a small (23% in average, n = 3) in[crease] in cAMP level within the first minute (Fig. [3]), after which the level was constant for the next [illegible]. From Fig. 2a, periods of 5 min duration [were] chosen as suitable for examining the dose–re[sponse] relationship. 0.01 mM SNP caused a sig[nificant] increase in the cGMP level and a maximum [wa]s reached with 1 mM (Fig. 3). This series con[firm]s that SNP has only a small effect on cAMP. [Inc]ubation for 5 min with 0.01 mM SNP caused a 20% increase in cAMP level (Fig. 3*b*) while the step from 1 to 10 mM resulted in a 13% decrease (n = 4).

Effect of SNP on OWF and SCC

In these expts. 1 mM SNP was used since this concentration gave maximal increase of the cGMP level and had negligible effect on the cAMP level. As seen from Table 1 1 mM SNP had no effect on OWF or SCC during a 20 min period. Neither did expts. with longer incubation periods or lower concentrations of SNP reveal any effects on OWF or SCC (data not shown).

Table 3. *cAMP response to AVT after 20 min preincubation in 1 mM SNP*

Symmetrical pieces were incubated for 20 min in solution with and without 1 mM SNP, then AVT (1 or 40 ng/ml) was added to both pieces and cAMP measured in both 5 and 30 min later. Values are mean ± S.E.

AVT ng/ml	min	pmole cAMP/mg dry weight +SNP	−SNP	+SNP/−SNP	n
1	5	1.16±0.09	1.28±0.12	0.93±0.07	4
	30	1.37±0.26	1.57±0.36	0.90±0.05 n.s.	4
40	5	6.47±1.11	7.68±2.24	0.90±0.08 n.s.	5
	30	[illegible]60±0.83	2.13±0.65	1.20±0.05 $0.01<P<0.02$	5

n.s. not significant.

Table 1 *Effect of SNP on OWF and SCC*

Paired halves were allowed to equilibrate and then 1 mM SNP was added to one half (time 0) OWF and SCC were re time 0 and 20 min and the change with time calculated as the difference between the values at the two times (Δ) difference between the changes of the control-half (Δ−) and the experimental half (Δ+) were calculated and taken a effect of SNP Values are mean ± S E.

Para-meter	SNP	Minute 0	20	Δ	Δ+ − Δ−	n
OWF	+	6 6±0 8	7 1±1 0	0 5±0.3	0.1±0.3	11
($\frac{\mu l\ H_2O}{h\ cm}$)	−	6 3±0 6	6.7±0 8	0 4±0 4		
SCC ($\mu A/cm^2$)	+	13 0±2.7	11 8±2 3	−1 1±0.5	0 1±0.3	
	−	13 3±2.5	12.0±2.[illegible]	−1.2±0.5		

the specificity of the cGMP antiserum was satisfactory The authenticity of the measured cGMP values was verified by carrying standards through the extraction and purification procedure and by internal standards and dilution curves of epithelial extracts

In one series (Table 3) only cAMP was measured and the more convenient boiling method (Johnsen & Nielsen 1978) was used with the modification that the homogenization was replaced by a mixing on a whirli-mixer In a control series it was found that mixing gave 4±1% ($n=6$) higher values than did homogenization

Measurements of SCC and OWF

The hydroosmotic response to AVT of R temporaria was found to be rather small compared to that of R esculenta 31 ng/ml AVT increased the OWF 50–100% in R. temporaria vs 700–800% in R esculenta. Therefore R esculenta was used for the measurement of OWF and SCC The R. esculentas were kept at room temperature with free access to water and food A few experiments confirmed that the SNP induced cGMP accumulation in R esculenta is similar in magnitude to the one in R temporaria

The OWF measurements were carried out in a chamber which allows the simultaneous measurements of SCC and OWF (SCC was measured according to the technique of Ussing & Zerahn (1951)) This chamber is improved from the one described by Lichtenstein & Leaf (1965) with an automatic recording device akin to that described by Ruphi et al (1972) In short the principle is that the outside (apical side) of the skin is pressed against a stainless steel net with a pressure of 2 cm H_2O the half chamber containing the outside bathing solution is closed except for an outlet consisting of a capillary tube The outside bathing solution is allowed to flow into the capillary tube whereby the light transmission of the tube changes markedly The transmission is recorded by a detection fork (Hafo) (consisting of a light emitting diode and a photosensitive transistor) the signal of which controls a motor driven syringe to keep the position of the meniscus constant The motor also drives a precision potentiometer which is used as a potential divider allowing the position of the syringe to be recorded continuously on a pen recorder The [illegible] of the recording then gives the flow rate (Fig 1).

The detection limit of the apparatus was determ[illegible] changing the volume of a closed half chamber by me[illegible] a 1 µl syringe and it was found to be better than 50 nl overall precision of the flow rate determination de[illegible] upon detection l ml recorder speed and size of th[illegible] driven syringe In the present experiments a 50 µl syr[illegible] (Hamilton) was used and the recorder speed was 2 [illegible] min this gave a precision better than 0 µl h cm when the flow was 5 µl h cm (typical basal flow) a precision better than 0.5 µl h cm when flow was 25 µl h cm (typical stimulated flow)

The inside bathing solution was the above mention[illegible] Ringer s solution and the outside bathing solution c[illegible] sisted of the same solution diluted 5-fold giving a grad[illegible] of 190 mosm across the skin

Material

Synthetic AVT the neurohypophysial hormone from a[illegible] phibia was obtained from Calbiochem Al_2O 90 act[illegible] neutral was obtained from Merck SNP (Merck) a[illegible] ways dissolved immediately before use ³H-cGMP (Ci/mmol) ¹⁴C-cAMP (0 76 Ci/mmol) and ¹²⁵I-cGMP (6 Ci/mmol) was obtained from Amersham cGMP antiser[illegible] was kindly supplied by J Arends, Statens Seruminstitu[illegible] Copenhagen

RESULTS

Paired symmetrical pieces were used for all expts throughout this paper in order to compensate fo[illegible] the rather large biological variations in frog skin

Effect of SNP on cGMP and cAMP

Basal level of cGMP was 14 2±1 7 fmol cGMP/mg dry weight (mean ±S E $n=16$)

Incubation with 0 1 mM SNP caused a marked increase in the cGMP level (F[illegible] the text for

Table. Responses to AVT after 20 min preincubation with 1 mM SNP

For the experiments, symmetrical skin halves were preincubated for 20 min in the presence and absence of 1 mM SNP. Then AVT (1 ng/ml) was added to both skin halves. The stimulation of OWF and SCC was read 60 min later (values in % increase = 100 (a-b)/b where b=level before stimulation and a = level 60 min after stimulation), and the ratios between the two halves calculated. Then a maximal stimulating dose of AVT (31 ng/ml) was added to both halves and the total stimulation of OWF and SCC read another 60 min later. The calculations were performed as mentioned above. Values are means ± S.E. With t-test it was tested whether the ratios (+SNP/−SNP) were different from 1.00

AVT dose	Parameter	Unstimulated level	% increase after AVT +SNP	−SNP	+SNP/−SNP	n
1 ng/ml	OWF	6.1±0.5 µl H_2O/(h cm²)	254±66	229 71	1.31±0.14 0.05<P<0.1	8
	SCC	13.9±2.7 µA/cm²	99±34	97±32	1.00±0.04	
31 ng/ml	OWF	6.3+0.6 µl H_2O/(h cm²)	485 114	423 97	1.14±0.03 0.005<P<0.01	6
	SCC	14.9±3.6 µA/cm²	154+55	195±87	0.87±0.08 0.1<P<0..	

The percentage effect of SNP on the AVT induced OWF was smaller in the skins where AVT induced the larger increase in OWF. This explains the discrepancy between the ratio of the means (254/229 = 1.11) and the mean of the ratios for the individual experiments (1.31).

...levels. After a lag period about 1 min the cGMP level rose approximately 10 times within the next 4 min, and more slowly the following 20 min. 0.1 mM SNP induced a small (23% in average, n=3) increase in cAMP level within the first minute (Fig. 3b), after which the level was constant for the next ... min. From Fig. 2 ... periods of 5 min duration were chosen as suitable for examining the dose–response relationship. 0.01 mM SNP caused a significant increase in the cGMP level and a maximum was reached with 1 mM (Fig. 3a). This series confirms that SNP has only a small effect on cAMP ... incubation for 5 min with 0.01 mM SNP caused a 20% increase in cAMP level (Fig. 3b), while the step from 1 to 10 mM resulted in a 13% decrease (n=4).

Effect of SNP on OWF and SCC

In these expts. 1 mM SNP was used, since this concentration gave maximal increase of the cGMP level and had negligible effect on the cAMP level. As seen from Table 1, 1 mM SNP had no effect on OWF or SCC during a 20 min period. Neither did expts. with longer incubation periods or lower concentrations of SNP reveal any effects on OWF or SCC (data not shown).

Table 3. cAMP response to AVT after 20 min preincubation in 1 mM SNP

Symmetrical pieces were incubated for 20 min in solution with and without 1 mM SNP, then AVT (1 or 40 ng/ml) was added to both pieces and cAMP measured in both 5 and 30 min later. Values are mean ± S.E.

AVT ng/ml	min	pmole cAMP/mg dry weight SNP	−SNP	+SNP/−SNP	n
1	5	1.16±0.09	1.28±0.12	0.93±0.07 n.s.	4
	30	1.37±0.26	1.57±0.36	0.90±0.05 n.s.	4
40	5	6.47±1.11	7.68±2.24	0.90±0.08 n.s.	5
	30	2.60±0.83	2.13±0.65	1.20±0.05 0.01<P<0.02	5

n.s. not significant

Effect of SNP on stimulation with AVT

To test whether SNP influenced the ability of ADH to stimulate OWF and SCC a series of expts were performed where the skins were preincubated for 20 min in the presence and absence of SNP After the preincubation a submaximal dose of AVT (1 ng/ml) was added and the SCC and OWF were recorded for 60 min Then a dose giving maximal stimulation of SCC (31 ng/ml) (Johnsen & Nielsen 1978) was added and the SCC and the OWF were recorded for another 60 min SNP had no significant influence on the AVT induced stimulation of SCC but it enhanced the stimulation of OWF moderately (Table 2) This potentiation was significant at the high concentration of AVT At the low concentration of AVT SNP was found to potentiate the AVT induced increase of OWF in 7 out of 8 expts in these 7 expts the mean value of the ratio between OWF in the presence and absence of SNP was 1 38±0 14 (0 025< P<0 05)

To make sure that 31 ng/ml AVT induces maximal stimulation of OWF and SCC the following series of 3 expts was performed At first the skins were stimulated by 31 ng/ml AVT and when a new steady state was reached the concentration of AVT was increased to 186 ng/ml This increase in the AVT concentration stimulated the SCC by 1 9 0 7 and 2 4% and the OWF by 2 8 0 and 0%

To investigate whether the effect of SNP on the AVT induced stimulation of OWF could be attributed to a change in the cAMP level the expts in Table 3 were performed Stimulation with AVT results in an increase of the cAMP level which reaches a maximum after 5 min thereafter it declines to a semi-steady state level at 30 min (Johnsen & Nielsen 1978) Since it is not known whether the magnitude of the physiological responses SCC and OWF depends on the peak or the steady state value of the cAMP level measurements were made 5 and 30 min after addition of AVT Preincubation with SNP had only a significant effect on the AVT stimulated cAMP accumulation in the series carried out at the high AVT concentration for 30 min where a slight potentiation by SNP was found (Table 3)

DISCUSSION

SNP stimulates cGMP accumulation in a number of tissues (Böhme et al 1978) In the present communication it is shown that SNP causes a mar[ked] increase in the cGMP level in the ho[le] epithelium of frog skin (Fig. 2*a* and Fi[g] whereas SNP has only a negligible effect on cAMP level (Fig 2*b* and Fig 3*b*) The SNP [in]duced cGMP accumulation in frog skin is comp[ara]ble in magnitude and concentration dependence [to] the one found in smooth muscle tissue (Böhme e[t al] 1978)

SNP per se has no effect on OWF or SCC (T[able] 1) indicating that intracellular cGMP is with[out] effect on either of these parameters But SNP h[as a] moderate potentiating effect on the AVT indu[ced] stimulation of OWF while it is without effect on stimulation of SCC (Table 2) SNP also enhan[ces] the elevation of the semi steady state level of cA[MP] induced by a dose of AVT (31 ng/ml) that gi[ves] maximal stimulation of SCC and OWF (Table 3 [It] is tempting to use this as an explanation of [the] potentiating effect of SNP on the OWF stimula[tion] by AVT But this is probably not the case si[nce] hormone doses exceeding those needed for ma[xi]mal physiological stimulation are generally capa[ble] of eliciting a further increase in the cAMP le[vel] (Robinson et al 1971) In toad bladder for examp[le] ADH concentrations higher than necessary to g[ive] maximal stimulation of SCC and OWF provo[ke] additional cAMP accumulation (Flores et al 197[5]) Thus it seems more likely that the potentiation [of] the response to AVT by SNP comes from interf[er]ence of cGMP or SNP with another step in t[he] stimulus response coupling than the stimulation [of] adenylate cyclase

Our results are in contrast to the findings of P[ic]cini & Paris (1973) that exogenous cGMP inhib[its] the stimulation of OWF by oxytocin in toad bladd[er] Unless this discrepancy is due to species diffe[r]ences the result of Piccini & Paris might be [a] nonspecific result of the high concentration [of] cGMP (10 mM) employed in their expts Sugita [et] al (1973) found a small potentiating effect of exog[e]nous cGMP on the SCC response to ADH in toa[d] bladder whereas we found a potentiating effect [of] SNP on the OWF response in frog skin

The conclusion to be drawn from the expts pre[]sented in this paper is that cGMP in contrast t[o] cAMP has no influence on OWF or SCC and pla[ys] no part in the regulation of SCC by AVT On th[e] other hand sodium nitroprusside moderately poten[]tiates the AVT stimulation of OWF this may be du[e] to the enhanced cGMP levels but it cannot b[e]

…is added that it is caused by an effect of SNP on … the parameters. Our results lends no support to … the general idea of opposing effects of cAMP and … cGMP (Goldberg et al. 1972). The idea of opposing effects of cAMP and cGMP was recently supported by the observation that carbamylcholine reduces the SCC and increases the cGMP level in toad bladder (Wiesmann et al. 1978, Sahib et al. 1978). Considering our results it seems more likely that the carbamylcholine induced increase in cGMP level does not induce the reduction of SCC. This possibility was also mentioned by Wiesmann et al. (1978) as they have suggested that both the decrease in SCC and increase in cGMP level induced by carbamylcholine are elicited by the entry of calcium into the cells.

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Functional properties of neurones in the parietal retroinsular cortex in awake monkey

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LEINONEN, L.: Functional properties of neurones in the parietal retroinsular cortex in awake monkey. Acta Physiol Scand 1980, 108: 381–384. Received 13 July 1979. ISSN 0001-6772. Department of Physiology, University of Helsinki, Finland.

The parietal retroinsular cortex of three awake behaving macaque monkeys was investigated using transdural microelectrode recording technique. All 73 cells identified responded to somatosensory stimuli. Most of the neurones (51) were activated by compression of the skin in an on, off or on-off fashion; these cells were unresponsive to light touching or stroking of the skin. The rest of the cells responded to light touching, rotation of a joint or palpation of a muscle belly. All body parts were represented in this area. Of the cells 30% responded to stimulation of both sides of the body. The results indicate that the parietal retroinsular cortex participates in the analysis of skin compression. This information is used, e.g. in the control of manipulative movements and in discrimination of supported weights. It is possible that ablation of the area examined would impair these functions.

Key words: Parietal lobe, somatosensory cortex, microelectrode recording

Area SII of the monkey buried in the medial wall of the Sylvian fissure was previously investigated with single cell recording technique by Whitsel et al. (1969). Their study did not include the most posterior part of the medial wall of the Sylvian fissure which they regarded as part of SII. However, there is some histological evidence (Pandya & Sanides 1973, Jones & Burton 1976) that the parietal retroinsular cortex in the caudal end of the fissure is architectonically different from SII. The aim of this study was to explore this part of the parietal cortex and to compare its functional properties with those documented from SII by Whitsel et al. (1969).

All cells in this study were responsive to somatosensory stimulation, but in contrast to cells in SII most of them did not respond to light touching or stroking of the skin but only to compression (causing clear deformation) of the skin.

MATERIAL AND METHODS

Recordings were made from four hemispheres of two adult male and one juvenile male Macaca speciosa monkeys weighing 6–8 kg. Results obtained from the same monkeys from area 7 and the temporoparietal association cortices are reported elsewhere (Leinonen 1980, Leinonen et al. in prep.). The discharges of each neurone isolated were observed during the monkey's tracking and fixating eye movements, when the monkey reached and grasped objects under visual or tactual guidance or when the monkey brought objects to the mouth. Each neurone was also tried to activate by light touch, pressure on the skin (compression of the skin between the underlying bone and the external pressure), palpation of muscle bellies, rotation of joints and various moving and stationary visual stimuli. Fig. 1 shows the area recorded and the histological map by Pandya & Sanides (1973) of the posterior part of the Sylvian fissure and temporal lobe.

RESULTS

All cells identified in the retroinsular cortex responded only to somatosensory stimulation. Properties of only one of the 74 cells studied remained unidentified (Table 1).

Cells responding to touch or pressure on the skin

8 of the 59 cells responsive to cutaneous stimulation were activated by light touch on the skin or blowing into the hair. Of the cells 51 responded only to compression of the skin (causing clear deformation), usually in an on, off or on–off fashion

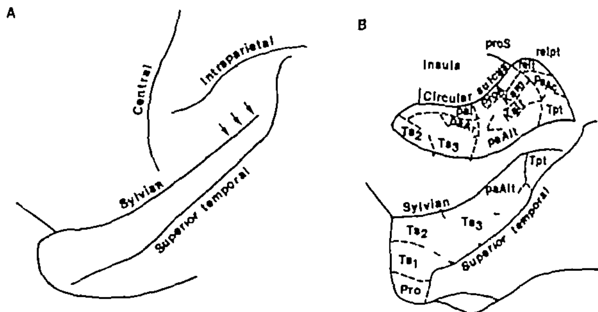


Fig 1 A The sulci of the parietal lobe and the recording site of the present work in the Sylvian fissure (arrows Cytoarchitectonic map by Pandya & Sanides (1973) of the posterior part of the Sylvian fissure and the superior tem gyrus Area relpt is the parietal retroinsular area recorded

(Fig 2) Some cells fired for several seconds during continual pressure on the skin (Fig 3) 37 cells had a receptive field on the hand or arm 5 cells on the shoulder neck or head 2 on the trunk and 15 on the legs only (Fig. 4) The receptive fields were rather large e g the whole hand whole foot all fingers whole arm Those cells that had a receptive field on the hand were usually active also when the monkey was manipulating an object or grooming

Cells responding to rotation of joints or palpation of muscles

Fourteen cells responded to forced or rapid dorsal flexion of fingers or wrist (5 cells) dorsal flexion of the ankle joint (? cells) or wrist and ankle joints (1 cell) to palpation of muscle bellies in the lower or upper arm (? cells) or legs (1 cell) to passive rotation of the back or neck and to palpation of the muscles of the neck or back (3 cells) (Fig 4)

Laterality

Of the 73 cells 51 had their receptive field on contralateral side of the body 22 cells were a vated by stimuli on either side of the body W the bilateral receptive fields were on the extrem the responses were usually equally strong to stim on the right and left sides

Referent body part

All parts of the body were represented in the a examined Most cells (48 out of 73) had a recept field on the hand or arm the rest on the should neck head trunk or legs

DISCUSSION

Area SII of the monkey buried in the Sylv fissure was studied with single cell recording tec

Table 1 *Classification of the cells in the pariet retroinsular cortex according to the type of effe tive stimuli*

	No of cells	
Cell responding to touch or pressure on the skin	59	[illegible]
Cell responding to palpation of muscles or rotation of joint	14	[illegible]
Not identified	1	1
Total	74	10[illegible]

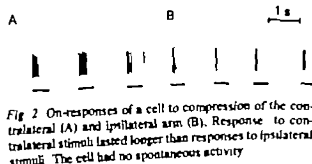


Fig 2 On-responses of a cell to compression of the contralateral (A) and ipsilateral arm (B). Response to contralateral stimuli lasted longer than responses to ipsilateral stimuli The cell had no spontaneous activity



Fig 3 Response of cell to compression of the contralateral forefinger The response lasted several seconds during stationary stimulus N off-responses were observed The cell had no spontaneous activity

nique by Whitsel et al (1969) This study did not include the most posterior part of the Sylvian fissure However Whitsel et al proposed that S II extends to the end of the fissure which view is also held by Woolsey (1958) and Roberts & Akert (1963). In their histological investigations Pandya & Sanides (1973) and Jones & Burton (1976) described a retroinsular area in the dorsal wall of the Sylvian fissure posterior to S II and lateral to area 7 The present study was conducted in this area The comparison of the results from the retroinsular area with those obtained from S II by Whitsel et al (1969) shows that the areas are functionally different. The areas are similar as far as all cells respond to somatosensory stimulation and all parts of the body are represented in both areas. The findings differ in the following respects. (1) In S II 87% of the cells responded to light touching or stroking of the skin and 13% were activated only by compression causing clear deformation of the skin whereas in the retroinsular area only 10% of the cells responded to light touch or stroking and most cells were activated only by compression of the skin or palpation of muscle bellies or rotation of joints (2) In S II 90% of the cells had bilateral receptive fields whereas in the retroinsular cortex most (70%) cells had only contralateral receptive fields.

Connections of the retroinsular cortex

Burton & Jones (1976) showed that the anterior and medial parts of the posterior nucleus of the thala-

Fig 4 Receptive fields (black) of all cells responsive to cutaneous stimulation. The receptive fields on the extremities always extended around the limb

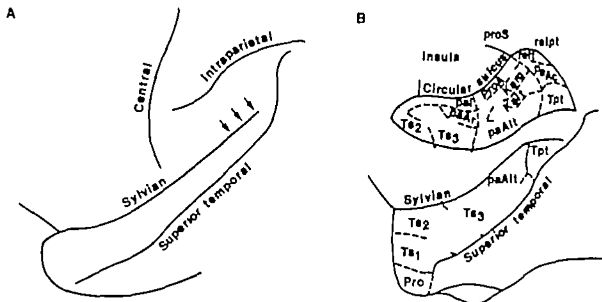


Fig 1 A The sulci of the parietal lobe and the recording site of the present work in the Sylvian fissure (arrows) B Cytoarchitectonic map by Pandya & Sanides (1973) of the posterior part of the Sylvian fissure and the superior temporal gyrus Area relpt is the parietal retroinsular area recorded

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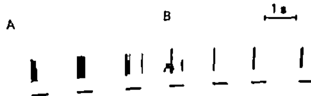


Fig 2 On-responses of a cell to compression of the contralateral (A) and ipsilateral arm (B) Responses to contralateral stimuli lasted longer than response to ipsilateral stimuli The cell had no spontaneous activity

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Table 1 *Clas ification f the cells in the parieto retr in ular c rtex acc rding t the type of effec ti e stimuli*

	No of cells	%
Cells responding to touch or pressure on the kin	59	80
Cells resp nding to palpation of muscles or rotation of joint	14	19
Not identified	1	1
Total	74	100

Diurnal fluctuation in striatal choline acetyltransferase activity and strain difference in brain protein content of the rat

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NORDBERG A & WAHLSTRÖM G Diurnal fluctuation in striatal choline acetyltransferase activity and strain difference in brain protein content of the rat. Acta Physiol Scand 1980, 108 385–388 Received 13 July 1979 ISSN 0001-6772. Department of Pharmacology University of Uppsala, Sweden and Department of Pharmacology University of Umeå, Sweden

Choline acetyltransferase (CAT) activity was measured in two brain regions of rat at different times of the day A diurnal fluctuation of the CAT activity w s found in the striatum with high activity during the light period and low activity during the dark period In the hippocampus+cortex the CAT activity remained constant. A marked difference in the brain protein content was found between two strains of Sprague-Dawley rats This finding emphasizes the importance of expressing enzyme activity both per g tissue and g protein especially when comparing data between different research groups

Key words. Choline acetyltransferase, diurnal fluctuation rat, brain regions strain

A diurnal fluctuation in the content of acetylcholine (ACh) in brain has been reported by several research groups (Friedman & Walker 1969 Hanin et al 1970 Saito 1971 Saito et al. 1975 Massarelli 1975) There are also data indicating that the enzyme choline acetyltransferase (CAT) responsible for the synthesis of ACh may fluctuate in activity with diurnal periodicity in the brain (Walker & Spooner 1975, Massarelli 1975). In the present study we present data indicating that the activity of CAT in the striatum of rat fluctuates diurnally while remaining constant in the hippocampus and cortex. In addition a marked difference in brain protein content was measured between two strains of rats

METHODS

Two strains of male Sprague-Dawley rats were used namely Nb/Han/Mol Møllegaard Ll, Skensved, Denmark (?-415 g) (strain A) and Mad/Han/Anticimex, Stockholm, Sweden (370–475 g) (strain B). The animals were kept 3/cage The artificial light which was the only light source was for strain A on an inverted schedule with light between 8 00 p m. and 8 00 a m For strain B the light was on between 6 00 a m and 6 00 p.m. Food and water were available ad libitum. For the study of the diurnal fluctuation in CAT activity only rats of strain B were used and they were kept under our diurnal lighting pattern for at least 2 months before they were killed.

At the time of sacrifice each rat was separately removed from the animal room and immediately killed in a room near by The animals were killed by decapitation. The brains were rapidly removed and put on an ice-cold glass plate. The striatum and the hippocampus + cortex were dissected essentially according to the method described by Glowinski & Iversen (1965) The tissues were homogenized in solution of NaCl 150 mM–EDTA 0.2 mM–butanol 1% (1 100 w/v) The activity of CAT was measured according to the method by Fonnum (1969) with some modifications (Glover & Green 1972, Aquilonius et al 1975). 10 µl of the homogenate were incubated with 10 µl phosphate buffer 20 mM (pH 7.0) containing ^{3}H acetyl-CoA (100 mCi/mmol) 140 µM choline (Ch) Cl 10 mM KCl 200 mM and physostigmine salicylate 0.2 mM for 10 min at 37°C The reaction was stopped by adding 10 µl 1 M HAc Radioactive ACh (^{3}H ACh) formed was extracted into 100 µl 3-heptanone containing 0 1 M K_4HgI_4 Fifty microliters of the organic layer were added to a scintillation coctail containing toluen, methanol Permablend 111 and the radioactivity was measured The protein content of the homogenates were measured according to Lowry et al (1951) Bovine albumin was used as standard.

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mus project to the parietal retroinsular area in the posterior part of the Sylvian fissure. They also stated that "Projections from the ventroposterior nucleus outline the boundaries of the second somatic sensory area in the upper bank of the lateral sulcus. The posterior boundary of S II appears to contain the representation of the hind limb and abuts mainly upon the retroinsular field and area 7 but does not overlap into either of these". The posterior nucleus of the thalamus is thus one possible source of somesthetic information received by the retroinsular area. Information may also come via SI and area 5 which project to the area studied (Jones & Powell 1970). The whole dorsal wall of the Sylvian fissure receives transcallosal fibers (Pandya et al. 1971) which is in accordance with the finding that 30% of the cells examined had bilateral receptive fields.

Role of the parietal retroinsular cortex

Burton & Jones (1976) have discussed the projection from the posterior thalamus to the parietal retroinsular cortex in the light of animal experiments and clinical findings on central mechanisms of pain. The present study revealed that most cells in the parietal retroinsular cortex responded only to rather intense pressing of the skin. However, if e.g. the monkey's withdrawal of the hand were considered as a criterium for a painful stimulus, the stimuli used were not painful.

Pandya & Sanides (1973) suggested that the parietal retroinsular field receives vestibular projections. Burton & Jones (1976) proposed that vestibular projections from the posterior thalamus terminate in the dysgranular insular area. The present study showed that all cells in the retroinsular cortex responded to somatosensory stimuli. It was not examined whether they would have responded also to vestibular stimuli. However, the results show that this area is not purely a vestibular projection field which agrees with the concept of Burton & Jones (1976) about the vestibular projection area.

In the parietal retroinsular area most cells examined responded to compression of the skin. Similar stimuli have been observed to activate cells of area 7 near the retroinsular area (Leinonen 1980). Cells in the bordering part of area 7 respond also to visual stimuli or are active during the monkey's own movements, most often during movements of the hands, e.g. grasping, manipulation and grooming. It is possible that information from the parietal retroinsular area is transferred to area 7 where it is used for the control of hand movements. The information analyzed in the parietal retroinsular area might also be used for discrimination of support weights, which ability has been shown to deteriorate after a damage in the posterior parietal cortex (Ruch et al. 1938).

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Table I. CAT activities in the striatum and hippocampus + cortex of 2 different strains of Sprague-Dawley rats

Mean value ± the standard error are given. n denotes number of rats. In all statistical comparisons strain A is compared with strain B

	nmol ACh/g tissue/min	nmol ACh/mg protein/min	mg protein/g tissue
Striatum			
Strain A	122.13 ± 4.75** n=9	3.19 ± 0.4 n=8	39.18 ± 2.10* n=8
Strain B	234.88 ± 15.97 n=9	3.68 ± 0.29 n=5	63.9 ± 3.02 n=7
Cortex + hippocampus			
Strain A	86.64 ± 2.67** n=9	1.48 ± 0.07 n=7	57.96 ± 1.95*** n=7
Strain B	119.16 ± 8.35 n=9	1.38 ± 0.11 n=5	74.86 ± 2.88 n=5

$P<0.01$, *** <0.001

killed by decapitation while no difference in ACh content was found following freezing in liquid nitrogen. Saito et al. (1975) also recorded higher ACh values in rats killed during the light period in comparison with the dark period but in contrast to Hanin et al. (1970) this diurnal rhythm in ACh content was found independent of the method of sacrifice. Recently it has been shown that killing animals with microwave irradiation is necessary in order to prevent post mortem changes in ACh levels (Stavinoha et al. 1973, Nordberg & Sundwall 1976). As far as we know no study concerning diurnal ACh fluctuation following this rapid method of killing has been presented.

In agreement with the findings concerning ACh we found in the present study a high CAT activity during the light period and a lower activity during the dark period. It is surprising to find this since CAT is thought to be non-rate limiting in the synthesis of ACh (Kuhar & Murrin 1978). But the fact that this diurnal fluctuation in CAT only could be found in the striatum and not in the cortex + hippocampus might be explained by the very rapid turnover and high concentration of ACh in the striatum in comparison with other brain regions (Nordberg 1977). Since the previous reports on changes in CAT activity mentioned in the introduction (Walker & Ikpoodia 1975, Massarelli 1975) are not given with sufficient details a comparison with the present material is not possible. In the abstract by Walker & Ikpoodia (1975) it is not stated at what time the light was switched off. Massarelli (1975) used the whole brain of the mouse. Recently data have been presented indicating a higher CAT activity in the cerebral cortex of humans who have died in the morning compared with during the night (Perry et al. 1977*a*, 1977*b*). Whether this finding represents a diurnal fluctuation in CAT activity is hard to decide on this material only since the status of the individuals before and at the time of death might influence the results and the number of patients in each time group is small.

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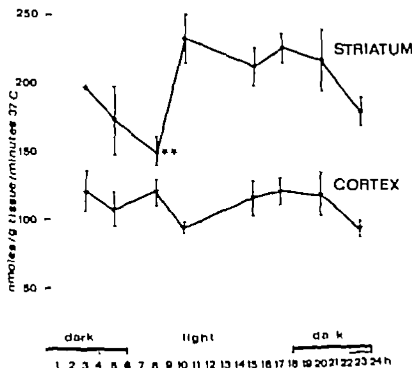


Fig 1 Diurnal fluctuation in CAT activity (nmol formed ACh/g tissue/min) in the striatum and cortex (including hippocampus) Each point represents the mean value of 4 rats. Vertical bars indicate S E $P<0.05$ $P<0.01$ in comparison with CAT activity at 10 a.m

RESULTS

Table 1 shows the activity of CAT in the striatum and cortex + hippocampus of two different strains of Sprague-Dawley rats The rats were killed between 1 h before and 2 h after the light had been switched off A significantly lower CAT activity expressed in nmol ACh formed/g brain tissue/min was found in the striatum and cortex + hippocampus of strain A in comparison with strain B In contrast no difference in enzyme activity was found between strain A and B when the activity was expressed in nmol/mg protein/min As shown in table 1 this finding can be explained by significantly lower protein content/g tissue in the striatum (−63%) and cortex + hippocampus (−29%) of strain A in comparison with strain B

Fig 1 shows the CAT activity (nmol/g/min) in the striatum and cortex + hippocampus of rats killed at different time of the day No significant difference in the CAT activity was found in the cortex + hippocampus However in the striatum a diurnal fluctuation in CAT activity was recorded A sharp rise in the enzyme activity was measured 4 h after the light had been switched on (at 10 a m) The CAT activity then was rather constant during the rest of the light period and dark period until 11 p m (5 h dark) when the CAT activity was significantly lower (−24%) in comparison with the activity at 1 p m The activity continued to be low during the rest of the dark period and at 2 h after the light had been switched on again (8 a m) the lowest CAT activity was measured (−35% in comparison with the value at 10 a m) Between 8 a m and 10 a m there was a marked increase in the CAT activity Thus the curve is essentially biphasic with a distinct maximum and minimum The same diurnal fluctuation in CAT was obtained when the enzyme activity was given in nmol ACh synthesized/mg protein/min No diurnal fluctuation in protein content of the brain was found

DISCUSSION

Hanin et al (1970) found a diurnal oscillation in the content of ACh of rat brain The ACh content was highest after 2 h light and lowest after 6 h darkness The fluctuation in the ACh content was only observed in grouped animals which had been under controlled environment with 12 h light and 12 h dark for at least 18 days It could only be observed in rat

Table I. *CAT activities in the striatum and hippocampus + cortex of 2 different strains of Sprague Dawley rats*

Mean value ± the standard error are given. n denotes number of rats. In all statistical comparisons strain A is compared with strain B

	nmol ACh/g tissue/min	nmol ACh/mg protein/min	mg protein/g tissue
Striatum			
Strain A	122.13 ± 4.75 *** n=9	3.19±0.24 n=8	39.18±2.10* n=8
Strain B	234.86 ± 15.97 n=9	3.68 ± 0.29 n=5	63.97±3.02 n=7
Cortex + hippocampus			
Strain A	86.64±2.67* n=9	1.48±0.07 n=7	57.96±1.95 n=7
Strain B	119.16 ± 8.35 n=9	1.38±0.11 n=5	74.86±2.88 n=5

* P<0.01 *** <0.001

killed by decapitation while no difference in ACh content was found following freezing in liquid nitrogen. Saito et al. (1975) also recorded higher ACh values in rats killed during the light period in comparison with the dark period but in contrast to Hanin et al. (1970) this diurnal rhythm in ACh content was found independent of the method of sacrifice. Recently it has been shown that killing animals with microwave irradiation is necessary in order to prevent post mortem changes in ACh levels (Stavinoha et al. 1973, Nordberg & Sundwall 1976). As far as we know no study concerning diurnal ACh fluctuation following this rapid method of killing has been presented.

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Capillary permeability in canine myocardium as determined by bolus injection residue detection

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HAUNSØ S., PAASKE, W. P., SEJRSEN, P. & AMTORP, O. Capillary permeability in canine myocardium as determined by bolus injection residue detection. Acta Physiol Scand 1980, 108: 389–397. Received 15 July 1979. ISSN 0001-6772. Department of Cardiology, Copenhagen County Hospital Gentofte, Department of Internal Medicine P, Rigshospitalet, and Institute of Medical Physiology B, University of Copenhagen, Denmark.

Capillary permeability of ^{51}Cr EDTA in the canine myocardium was determined by applying (A) the single injection, external registration method, and (B) the local tissue clearance method to the intact dog heart of open-chest anesthetized dogs. (A) ^{51}Cr EDTA was administered into the left anterior descending coronary artery as a bolus injection, and the response curve was recorded by external registration. The capillary diffusion capacity (the permeability-surface area product) for ^{51}Cr EDTA amounted to 32.5 ml/100 g min at capillary extraction of 0.40 and a plasma flow of 73.3 ml/100 g min. The diffusional permeability coefficient of ^{51}Cr EDTA was calculated to 1.08×10^{-5} cm/s, which indicates that the permeability of the capillaries in the myocardium for ^{51}Cr-EDTA is similar to that of continuous capillaries in other tissues. (B) ^{51}Cr EDTA (3–100 µl) was injected at a depth of 5 mm into the myocardium of the left ventricular free wall and the residue curve was recorded. The capillary extraction, as determined by the tissue clearance method, was calculated to values 6 times smaller than determined with the single injection, residue detection method. This unreasonably low extraction was probably due to methodological errors inherent to the tissue clearance technique.

Key words: Capillary permeability in canine myocardium

The filtration-permeability pore model of transcapillary hydrodynamic flow of solvent and diffusive flux of solute was proposed by Pappenheimer and co-workers as a result of a theoretical analysis and the data obtained with the osmotic transient method during filtration (Pappenheimer & Soto-Rivera 1948) and isogravimetry (Pappenheimer, Renkin & Borrero 1951). From the theories of equivalent pore (Ferry 1936, & Solomon 1968) and restricted diffusion (Fåhræus 1922, Bohlin 1960, Bean 1972, Bock & Schultz 1977, Vermory et al. 1973, Paine & Scherr 1975) it was deduced that circular cylindrical water-filled pores of 30 Å radius or—alternatively—parallel plate channels with a slit width of 37 Å could account for the experimental results obtained in capillaries of the continuous type (Mann 1965). The interendothelial slit (Karnovsky 1967, 1968) is generally believed to be the morphological basis for this small pore system. The osmotic transient method is, however, complicated with corrections for the osmotic reflection coefficient (Staverman 1951) and the fact that solvent (water) passes the whole capillary surface area leads to further obstacles as the analysis considers the pores to be the sole transcapillary exchange pathway (Perl 1971, 1973).

A number of studies of capillary diffusive permeability with methods based solely on indicator diffusion principles have consistently provided experimental evidence for a much larger equivalent pore radius of this small pore system (for survey see Paaske 1979). The recently discovered transendothelial channels of fused vesicles (Simionescu, Simionescu & Palade 1975, 1976) appear to be a

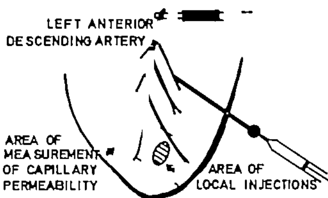


Fig 1 Schematic illustration showing the preparation of the left descending coronary artery for Cr EDTA and ^{13}I albumin administration

morphological system that might prove to be the transendothelial pathway for permeation of smaller hydrophilic substances and possibly proteins (Paaske 1976 Paaske & Sejrsen 1977)

The present study was consequently undertaken in order to reexamine the myocardial capillary permeability of the commonly used hydrophilic indicator molecule ^{51}Cr-ethylenediamine-tetraacetate (Cr EDTA molecular weight 341.2 molecular radius 4 7 Å free diffusion coefficient in water at 37°C 0 70×10^{-} cm²/s) with an alternative indicator diffusion technique i e the single injection residue detection method (Sejrsen 1970 1979) This method has proved well suited to measurement of capillary permeability in other tissues with continuous capillaries (Paaske & Nielsen 1973 1976 Paaske 1976 Paaske 1977*a b & c* Paaske & Sejrsen 1977 Sejrsen 1979) Further a series of investigations were carried out with a local tissue clearance technique (Lassen & Trap-Jensen 1968*a & b*) as this method a priori seemed useful for the present purposes

METHODS

Animal preparation The experiment were performed on 6 mongrel dogs weighing 22 to 27 kg Anesthesia wa introduced by thiomebumal sodium 12.5 mg/kg i The dogs were intubated and artificially ventilated with oxygen and nitrous oxide I v injections of fentanyl (Haldid®) 0 1 mg and pancuronibromidum (Pavulon) 2 mg were gi en when required A pigtail catheter was introduced through the left femoral artery and the tip placed in the ascending aorta. A Swan-Ganz flow directed thermodilution catheter was inserted through the right external jugular vein with the tip placed in the pulmonary artery A left thoracotomy was made and the pericardium was incised and sutured to the thoracic wall The left anterior descending coronary artery (LAD) was isolated about 2 cm from its origin and a silk snare was placed around the artery to be used for occlusion A polyethylene catheter was inserted into a small proximal ventricular branch of LAD (Fig 1) and its tip was positioned just inside LAD After the operative procedure the dogs were heparinized (400 U/kg)

Throughout the experiments were recorded Aortic and pulmonary blood pressures electrocardiogram, body temperature arterial oxygen saturation oxygen and carbon dioxide tensions, pH hematocrit values and cardiac output

Experimental procedure

A *The single injection residue detection experiments* 20–30 µl Cr EDTA (New England Nuclear 25 mCi/100 µl) was injected into LAD as a bolus through the polyethylene catheter The injection lasted about 1 s during which time LAD was occluded proximally by the silk snare A one inch NaI (Tl) scintillation detector located 5 cm above the heart recorded the activity The detector was connected to a universal printing gamma spectrometer (Meditronic Denmark) with an energy window of acceptance adjusted symmetrically around 320 keV photo peak of Cr The sampling integration time was 1 s.

B *The tissue clearance experiments* 3—100 µl ^{51}Cr-EDTA was injected at a depth of 5 mm into the myocardium of the left ventricular wall using a 0 5 mm needle. The thickness of the ventricular wall of the site of injections was around 11 mm The activity was recorded as indicated under A using a sampling integration time of 20 s

C *Measurement of regional myocardial blood flow* was performed with the local ^{133}Xenon washout technique 5 µl ^{133}Xenon (the Radiochemical Centre Amersham, England 10 mCi/ml) in isotonic saline was injected as under B The gammaradiation of ^{133}Xenon was recorded by a NaI (Tl) scintillation detector 5 cm inside the opening of a lead collimator The detector placed 10 cm above the heart was adjusted to the 81 keV photopeak of ^{133}Xenon The sampling integration time was 5 s

D *Measurement of intravascular plasma volume* was performed by bolus injection of 30 µl ^{131}I Human serum

Table 1 *Blood gas and hemodynamic parameters of 6 dogs measured before and recurrently during each expt*

Results are expressed as mean values ± S D *n* = number of observation

P_{aO_2} (mmHg)	145_36	(19)
P_{aCO_2} (mmHg)	32±9	(n 19)
pH	7 39 0 10	(19)
Hematocrit (per cent)	39_4	(n 19)
Heart rate (be t min)	1 4±43	(n 19)
Aortic pressure (mean) (mmHg)	104±13	(19)
Pulmonary arterial pressure (mean) (mmHg)	1 _4	(19)
Cardiac output (liter min)	4 7_1	(16)

Table 2. Capillary extraction, E, and permeability-surface area product, PS (capillary diffusion capacity) of ^{51}Cr-EDTA in the left ventricle of the canine myocardium

Plasma flow was calculated from the intravascular transit after curve resolution and compared to plasma flow calculated from the ^{133}Xenon washout curve. Hct denotes hematocrit corresponding to each expt.

Expt. no.	E (^{51}Cr EDTA) %	Plasma flow (ml/100 g min): Calculated from residue curve of ^{51}Cr-EDTA	Plasma flow (ml/100 g min): Calculated from ^{133}Xenon washout	Hct fraction	PS (ml/100 g min)
	0 469	69 4	86.0	0 360	39 1
	0 47?	57 1	86 0	0 360	32.5
	0 295	78 5	86 0	0 360	24.5
	0 493	63 4	63 4	0.370	38 4
	0 409	76 8	83.8	0 360	36 0
	0 271	97.0	83.8	0 360	27.6
	0 375	71 1	70.[illegible]	0 440	29 7
	0 358	73 3	79 9	0 373	32 5
±S D	0 088	12.8	9 2	0.030	5 6

[illegible] (Kyller 0.4 mCi/ml) as described under A. The spectrometer was adjusted around the 364 KeV peak of [illegible]. The sampling integration time was 0 5 s.

E. *Measurement of recirculation* was performed following injection of 30µl ^{51}Cr EDTA into the cavity of the left atrium, and activity was recorded as indicated under A.

Calculations

A. *The single injection residue detection experiments.* The capillary extraction and capillary permeability—surface area product (the capillary diffusion capacity) was calculated as described by Bergsen (1970, 1979) using the computerized technique of Paaske & Nielsen (1976). The highest recorded count value which occurred at peak time was taken as a relative measurement of the total injected dose. The part of the curve recorded from 20 to 50 s after the bolus injection was retropolated monoexponentially to peak time using the 'least square' regression procedure. The numerical value of this function was solved for each [illegible] and subtracted from the experimentally recorded curve (corrected for background) to give the intravascular transit function. The value of the monoexponentially extrapolated curve at peak time expressed as a fraction of the maximum count value gave capillary extraction, E. The permeability-surface area product, PS, was calculated as PS = −f (pl)$_{c}$ K ln (1−E) (ml/100 g min) (Renkin 1959) where f(pl)$_{c}$ is plasma flow and K is a constant for converting ml of plasma to ml of plasma water taking the molecular charge into consideration. For ^{51}Cr-EDTA a K value of 0.89 was used (Lassen & Trap-Jensen 1968 *b*). f(pl)$_{c}$ was calculated as f(pl)$_{c}$ = (1/t(iv)$_{c}$) V(iv)$_{blood}$ (1−Hct) 100 (ml/100 g min) (Kety 1951), where t(iv)$_{c}$—the intravascular mean transit time for the indicator—was calculated as area/height (Zierler 1965) of the intravascular curve obtained by retropolation and subtraction. V(iv)$_{blood}$ is the intravascular blood volume of the organ, and Hct is hematocrit value.

B. *The tissue clearance experiments.* Capillary extraction, E, was calculated according to Lassen & Trap-Jensen (1968 *b*): $E_{tc} = \lambda_{tc} \cdot k_{tc}/f(pl)$, where λ_{tc} is the tissue to blood partition coefficient of ^{51}Cr EDTA in ml/g (as a reasonable estimate 20 ml/100 g was used), and k_{tc} (min^{-1}) is the fractional removal rate of indicator from the labelled area. k_{tc} was calculated by regression analysing using 'least square' technique on the recorded count figures.

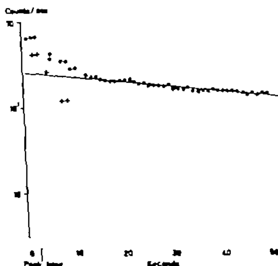


Fig. 2. Curve measured by residue detection after bolus injection of 30 µl ^{51}Cr EDTA. The dots denote the recorded count values corrected for background activity. The monoexponential regression line as calculated by the 'least square' method in the time interval from 20 to 50 s after the bolus injection is shown. Crosses signify the intravascular transit curve obtained by subtraction of the regression line from the original count values (dots).

Table 3 *Results obtained from the local tissue clearance expts using different volumes of ⁵¹Cr EDTA*

The fractional removal rate constant k_{te} from the labelled area, was calculated by regression analysing using least square technique on the recorded count figures

Expt no	Intramyocardial injected volume (µl) of Cr EDTA		k_{te} (min)
8			0.365
9	3		0 183
10	3		0.358
11	3		0 175
12	3		0 42
13	5		0 201
14	5		0 353
15	10		0.317
16	10		0 227
17	100		0 271
$n=10$		x̄	0.261
		± S D	0 074

C. *Calculation of regional myocardial plasma flow* $f(pl)_{Xe}$, was made from $f(pl)_{Xe}=k_{Xe}\ \lambda_{Xe}$ (1 Hct) 100 (ml/100 g min) where k_{Xe} is the slope (min) of the recorded washout curve of ¹³³Xenon and λ_{Xe} is the tissue to blood partition coefficient of ¹³³Xe (Kety 1951) λ_{Xe} was estimated in individual experiments in accordance to measured hematocrit values (Tonnesen & Sejrsen 1967)

D *The int avascula plasma olume* $V(iv)_{pl}$ was calculated from the equation $V(iv)_{pl}=f(pl)_{Xe}$ t(i $)_{alb}$ where I(iv)alb=area/height of the recorded curve of the intravascular indicator ¹³¹I albumin

E. *Determination of recirculation* was performed by direct recording of activity in the field following bolus injection of Cr EDTA into the left atrium

RESULTS

Variables characterising the physiological state of the preparation during the experiments are shown in Table 1

A *The single injection residue detection experiments* The results are tabulated in Table ? A representative example of the background corrected externally recorded curve and its resolution is presented in Fig ? The bolus injection was performed at time zero The initial rapid increase in recorded activity reached a maximum value of 666 counts/s at peak time and was followed by a fa decline A fairly monoexponential curve was obtained between 20 and 50 s In this time interval regression analysis was performed by means of the least square method By extrapolation of the curve section obtained from 20 s to 50 s a value of ?498 counts/s was obtained at peak time The slope k of the later curve section was −0 0136 s⁻¹ with standard deviation S D of 0 0009 s⁻¹ The capillary extraction E was (?498/6669)=0.375 The mean transit time I(iv) of the intravascular transfer function gave a plasma flow of 71 1 ml/100 g min PS then became PS=−71 1 0 89 ln(1−0.375)= 29 7 ml/100 g min

B *The tissue clearance experiments* k_{te} values and injection volumes are shown in Table 3 The average capillary extraction E was calculated to 0 067—a very low figure as compared to the results obtained with other independent methods—see discussion

C *Regional myocardial plasma flow* as determined with ¹³³Xenon from areas subjected to measurements of capillary permeability is given in Table 2

D *The intravascular plasma volume appears from Table 4*

E *Recirculation* of ⁵¹Cr EDTA was found to be 1–3 % of the injected dose

DISCUSSION

The theoretical basis for the single injection residue detection method has been given by Sejrsen

Table 4 *Capillary blood volume V(iv)blood per 100 g of tissue and the intravascular plasma volume V(iv)plasma calculated following direct determination of the intravascular mean transit time I(iv) from the area and height of the response curve of I-albumin*

f(pl) denotes regional plasma flow as determined by ¹³³Xenon washout Hct denotes hematocrit

Expt. no	I(iv)I-albumin (s.)	f(pl)Xe (ml/100 g min)	V(iv)plasma (ml/100 g)	V(iv)blood (ml/100 g)	Hct fraction
18	5 0	93.3	7 8	13 9	0 443
19	5 0	93.3	7 8	13 9	0 443
20	4 6	93.3	7	1 .8	0 443
x̄			7 6	13 6	

Table 5. Permeability coefficients (P) of capillaries in the heart for sucrose, ⁵¹Cr EDTA and inulin from various sources measured with different techniques

ID indicator diffusion method, OT osmotic transient method, SI single injection, residue detection method, TC tissue clearance method, TU tissue uptake method

Test substance	P 10⁵ (cm/s)	Method	Reference
Sucrose	11 0	TU	Schafer & Johnson 1964
Sucrose	3 9	TU	Vargas & Johnson 1964
Sucrose	0 8	ID	Alvarez & Yudilevich 1969
Sucrose	1 73	ID	Ziegler & Goresky 1971
Sucrose	1 9	OT	Bassingthwaighte et al 1975
Sucrose	1.2	ID	Bassingthwaighte et al 1975
Sucrose	1.22	ID	Laughlin & Duran 1975
Sucrose	2.2 3 3	ID	Rose & Goresky 1976
Sucrose	1.8	ID	Grabowsky & Bassingthwaighte 1976
Sucrose	0 87	ID	Harris et al. 1978
⁵¹Cr EDTA	*1 08*	*SI*	*Present study*
Inulin	0.4	TU	Schafer & Johnson 1964
Inulin	0 54	TU	Vargas & Johnson 1964
Inulin	0 27	ID	Alvarez & Yudilevich 1969
Inulin	0.58	OT	Bassingthwaighte et al. 1975
Inulin	0 36	ID	Laughlin & Duran 1975
Inulin	0.2	ID	Grabowsky & Bassingthwaighte 1976
Inulin	0 51	TU	Wittmers et al. 1976

1978, 1979) and Paaske (1979) and it must be emphasized that only one indicator is necessary in order to obtain the permeability-surface area product (PS). The problem of Taylor diffusion (Lassen & Crone 1970) is eliminated as an intravascular co-tracer is not necessary. The technique has earlier been applied to a multiple inlet, multiple outlet recirculating system using the technique of bolus injection under stopped flow conditions (cutaneous tissue, Paaske 1976). In a modified form the method was applied earlier to the heart by Parker et al. (1976). These authors simultaneously assessed myocardial blood flow and metabolism using cyclotrone produced ¹⁵O-water and ¹⁵O-Hemoglobin and external registration with a positron scintillation camera. The same experimental approach has been performed on the brain by Eichling et al. (1974) and Raichle et al (1976). Guller et al. (1975) determined myocardial permeability of sodium with the indicator diffusion method. The authors further analyzed the detected residue curves of sodium in accordance with single injection residue detection principles. They found that this method provided unreliable and inconsistent estimates of capillary extraction and PS. Sodium is a highly permeable substance and the difference between the rate constant of the intravascular (transmitted) and the extracted fraction of a bolus will be too small to distinguish reliably between the transfer functions. For hydrophilic molecules in the order of magnitude as ⁵¹Cr EDTA the present experimental data indicate that such problems are not encountered.

In the present study PS was 32.5 ml/100 g min (Table 2). From generally accepted estimates of capillary surface area, S, of 500 cm²/g (Bassingthwaighte et al 1974) P can be calculated to 1.08×10^{-5} cm/s. This figure is in good agreement with results found earlier with the indicator diffusion method (Table 5 and 6) using sucrose as indicator. ⁵¹Cr EDTA and sucrose have the same permeation and diffusion characteristics for all practical purposes. Capillary permeability of the myocardial capillaries for ⁵¹Cr EDTA is essentially the same as the capillary permeability of ⁵¹Cr EDTA in other organs and tissues with similar capillary morphology (skin, adipose tissue, skeletal muscle, Table 6). The present study consequently supports the hypothesis suggested earlier (Paaske 1976) that capillaries of continuous type exhibit similar permeation characteristics regardless of the tissue in which they are located. This view is supported by the findings of a number of studies (Table 6) that the ratio P/D (D = free diffusion coefficient in water) is constant, not only for the various smaller indicators but also from organ to organ.

The ratio of free diffusion coefficients between sucrose or ⁵¹Cr EDTA and inulin is about 3.2. The concept restricted diffusion implies a deviation of

Table 3 *Results obtained from the local tissue clearance expts using different volumes of ⁵¹Cr EDTA*

The fractional removal rate constant k_{Xe}, from the labelled area, was calculated by regression analysing using least square technique on the recorded count figures

Expt no	Intramyocardial injected volume (µl) of ⁵¹Cr EDTA		k_{Xe} (min⁻¹)
8	2		0 365
9	3		0 183
10	3		0.358
11	3		0 175
12	3		0.242
13	5		0.201
14	5		0.353
15	10		0.317
16	10		0.227
17	100		0.271
$n=10$		$\bar{x}$	0 261
		± S D	0 074

C *Calculation of regional myocardial plasma flow* $f(pl)_{Xe}$ was made from $f(pl)_{Xe}=k_{Xe}\ \lambda_{Xe}$ (1 Hct) 100 (ml/100 g min) where k_{Xe} is the slope (min⁻¹) of the recorded washout curve of ¹³³Xenon and λ_{Xe} is the tissue to blood partition coefficient of ¹³³Xe (Kety 1951) λ_{Xe} was estimated in individual experiments in accordance to measured hematocrit values (Tønnesen & Sejrsen 1967)

D *The intravascular plasma volume* $V(iv)_{pl}$ was calculated from the equation. $V(iv)_{pl}=f(pl)_{Xe}\ \bar{t}(iv)_{alb}$ where $\bar{t}(iv)alb$=area/height of the recorded curve of the intravascular indicator ¹³¹I-albumin

E *Determination of recirculation* was performed by direct recording of activity in the field following bolus injection of ⁵¹Cr EDTA into the left atrium

RESULTS

Variables characterising the physiological state of the preparation during the experiments are shown in Table 1

A *The single injection residue detection experiments* The results are tabulated in Table 2. A representative example of the background corrected externally recorded curve and its resolution is presented in Fig ? The bolus injection was performed at time zero The initial rapid increase in recorded activity reached a maximum value of 66?? counts/s at peak time and was followed by a fast decline A fairly monoexponential curve was obtained between 20 and 50 s. In this time interval regression analysis was performed by means of the least square method By extrapolation of the curve section obtained from 20 s to 50 s a value of ?49? counts/s was obtained at peak time The slope k of the later curve section was −0 0136 s⁻¹ with a standard deviation S D of 0 0009 s⁻¹ The capillary extraction E was (?498/6 669)=0.375 The mean transit time $\bar{t}$(iv) of the intravascular transfer function gave a plasma flow of 71 1 ml/100 g min PS then became PS=−71 1 0 89 ln(1−0.375) ?9 7 ml/100 g min

B *The tissue clearance experiments* k_{Xe} values and injection volumes are shown in Table 3 The average capillary extraction E was calculated to 0 067—a very low figure as compared to the results obtained with other independent methods—see discussion

C *Regional myocardial plasma flow* as determined with ¹³³Xenon from areas subjected to measurements of capillary permeability is given in Table 2

D *The intravascular plasma volume appears from Table 4*

E *Recirculation* of ⁵¹Cr EDTA was found to be 1–3 % of the injected dose

DISCUSSION

The theoretical basis for the single injection residue detection method has been given by Sejrsen

Table 4 *Capillary blood volume $V(iv)_{blood}$ per 100 g of tissue and the intravascular plasma volume $V(iv)_{plasma}$ calculated following direct determination of the intravascular mean transit time $\bar{t}(iv)$ from the area and height of the response curve of ¹³¹I-albumin*

f(pl) denotes regional plasma flow as determined by ¹³³Xenon washout Hct denotes hematocrit

Expt. no	$\bar{t}(iv)_{^{131}I\text{-albumin}}$ (s.)	$f(pl)_{^{133}Xe}$ (ml/100 g min)	$V(iv)_{plasma}$ (ml/100 g)	$V(iv)_{blood}$ (ml/100 g)	Hct fraction
18	5 0	93.3	7 8	13 9	0 443
19	5 0	93.3	7 8	13 9	0.443
20	4 6	93.3	7 2	12.8	0 443
		$\bar{x}$	7 6	13 6	

than could be expected. In some trials very small volumes of indicator (Table 3) were injected but these expts. gave essentially the same results as expts. with larger volumes. These findings might be accounted for if the interstitial space does not behave as a well mixed compartment with respect to the indicator so that concentration gradients exist in the region (Page 1963). Local volume expansion resulting in larger diffusion distances due to mechanical separation of the capillaries might play a pronounced contributory role.

The regional myocardial blood flow as determined from the kinetic analysis of the single injection, residue detection expts. and ^{133}Xenon local clearance technique did not differ significantly (p>0.10, paired t-test). The good correspondence between these two independent blood flow measurements supports the assumption of reasonable data analysis in the ^{51}Cr EDTA expts. The intravascular plasma volume was found to be 7.6 ml/100 g. This is in good agreement with the values of 5.7 and 7.0 ml/100 g found by Ziegler & Goresky (1971) and Rose & Goresky (1976) using sucrose as indicator. An estimate of an intravascular plasma volume of 7–8 ml/100 g seems to be reasonably correct as the intravascular curve of ^{51}Cr EDTA expts. corresponded very well to the shape of the albumin curves.

In conclusion: (1) the capillary permeability of the myocardium of ^{51}Cr EDTA ($P=1.08\times10^{-6}$ cm/s) is similar to the capillary permeability of other tissues with continuous capillaries. This supports the hypothesis that the permeability of continuous capillaries is the same regardless of the tissue in which they are located. (2) The pore size of myocardial capillaries is as yet unsettled but if the theory of an equal (identical) permeability of continuous capillaries holds true, the pores in the myocardium is larger (radius larger than 100 Å) than conventionally estimated from the Pappenheimer Karnovsky concept of a 37 Å slit width. (3) The single injection, residue detection method provides a useful and simple technique for measurement of capillary permeability in the heart. (4) The tissue clearance method systematically gives estimates of capillary extraction 6 times smaller than expected and cannot be recommended for measurement of capillary permeability in the heart.

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Table 6 *Permeability coefficient P of sucrose ⁵¹Cr EDTA ⁵⁷Co-B12 and Inulin in organs with continuous capillaries*

Abbreviations as under Table 5

Tissue	Indicator	P 10⁵ (cm/s)	Method	Reference
Skeletal muscle	Sucrose	0 74	ID	Crone 1963 *a & b*
	Sucrose	0 84	ID	Trap-Jensen & Lassen 1970
	Cr EDTA	0.85	ID	Trap-Jensen & Lassen 1970
	Cr EDTA	0 94	ID	Lassen & Trap-Jensen 1970
	Cr EDTA	0.89	ID	Trap-Jensen 1970
	Cr EDTA	0 78	ID	Trap-Jensen & Lassen 1971
	Cr EDTA	1 02	SI	Paaske 1977*c*
	EDTA	1 30	ID	Rippe & Grega 1978
	EDTA	1 35	ID	Rippe et al 1978
	Cr EDTA	1 16	ID	Sejrsen 1979
	Cr EDTA	1.06	SI	Sejrsen 1979
	⁵⁷Co-B12	0.59	SI	Paaske 1977*c*
	Inulin	0 26	ID	Crone 1963*a&b*
	Inulin	0 09	ID	Trap-Jensen & Lassen 1970
	Inulin	0 12	ID	Trap-Jensen & Lassen 1971
	¹⁴C Inulin	0 20	SI	Paaske & Sejrsen 1977
Adipose tissue	⁵¹Cr EDTA	0 95	SI	Paaske & Nielsen 1973 1976
	Sucrose	0 95	ID	Linde et al 1974
	⁵⁷Co-B12	0.52	SI	Paaske 1977*b*
Cutaneous tissue	⁵¹Cr EDTA	0 88	SI	Paaske 1976
	⁵⁷Co-B1	0 56	SI	Paaske 1977*a*

the ratio between the permeability coefficients from the ratio between the free diffusion coefficients The ratio of permeability coefficients between sucrose and inulin (Table 5) using the indicator diffusion method does not provide definite answer to the problem of whether restricted diffusion occurs in the heart for inulin as compared to the smaller indicators Also other techniques provide contradictory results The indicator diffusion experiments of Alvarez & Yudilevich (1969) provided evidence for absence of restricted diffusion for inulin as compared to sucrose ($P_{(sucrose)}/P_{(inulin)}$=2 9) whereas Laughlin & Diana (1975) found slight restricted diffusion ($P_{(sucrose)}/P_{(inulin)}$=3 4) corresponding to pores with a radius of 70–100 Å Determinations of the osmotic reflection coefficients by Vargas & Johnson (1966) resulted in a pore radius estimate of 35 Å Using the molecular sieving method Artursson Groth & Grotte (1972) found evidence for a pore radius of about 60 Å Essentially the same value (radius 55 Å) was found by Bassingthwaighte et al (1975) using determinations of the osmotic reflection coefficients *The presence of much larger* pores was suggested by Duran & Yudilevich (1978) based on experiments with sodium and glucose The diffusional water permeability P is about 60×10⁻⁵ cm/s (Rose et al 1977) and the hydraulic water permeability P_f is about 12 500×10⁻⁵ cm/s (Vargas & Johnson 1964) The ratio P_f/P is consequently 208 which corresponds to a pore radius estimate of some 50 Å

Results with the tissue uptake and osmotic transient methods cannot be considered reliable due to serious methodological problems inherent to these techniques The analysis of the indicator diffusion methods is based on assumptions of ideal Krogh cylinder behaviour of the microvasculature Rose & Goresky (1976) found heterogeneity of capillary transit times in the intact dog heart probably due to a large number of discrete parallel pathways. The influence of heterogeneity on the calculation of P from the conventional equations is a completely unsolved problem

The tissue clearance method has provided reasonable results from exercising skeletal muscle (Lassen & Trap-Jensen 1968*a*) of the same magnitude as those obtained with the indicator diffusion methods In the present series the extraction values were clearly underestimated with a factor of about 6 when compared to the other method employed i e the rate constants of the clearance from the interstitium to bl ere much smaller

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Effects of calcium and pH on the mechanical performance of heart muscle in the frog *Rana temporaria* during anoxia and subsequent recovery

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LAGERSTRAND G & POUPA O. Effects of calcium and pH on the mechanical performance of heart muscle in the frog, *Rana temporaria* during anoxia and subsequent recovery. Acta Physiol Scand 1980, 108, 399–404. Received 17 July 1979. ISSN 0001-6772. Department of Zoophysiology and Department of Clinical Physiology, University of Göteborg, Sweden.

Heart ventricular muscle strips from *Rana temporaria* recover their isometric contractile tension completely within 20 min of reoxygenation following an anoxic period of 60 min in physiological solution at pH 7.6. Corresponding recovery at pH 6.6 is only 43% of pretreatment values. High calcium concentration during the period of anoxia at pH 6.6 and subsequent recovery returns contractile tension to almost pre-anoxic values within 1 h. If, however, the calcium concentration is increased at the moment of reoxygenation, contractile tension is restored even faster than if high calcium levels were present during anoxia. The loss of contractile tension caused by anoxia is the same at both pH 7.6 and 6.6 with the same calcium concentration.

Comparison of the velocity parameters between high and low calcium experiments always shows a greater difference for the contraction velocities than for the corresponding relaxation velocities, independently of the pH. The quotient of these two velocities is used as an index of their relative rate of change. The results are interpreted in terms of calcium and hydrogen ion competition at various subcellular structures and the different influences these ions may have on contractile tension and both contraction and relaxation velocities.

Key words: Anoxia, reoxygenation, pH, Ca^{++}, frog, isometric contractile tension, contraction velocity, relaxation velocity

The recovery of contractile tension which occurs during reoxygenation after a temporary nitrogen induced hypoxia in frog ventricular cardiac muscle strips, has been shown to be more pH-sensitive than the decay of tension, which occurs during the preceding anaerobic period (Poupa & Gesser 1975).

Competition between calcium and hydrogen ions has been shown to occur at several cellular and subcellular levels (see Katz & Hecht 1969, Katz 1970, Williamson et al. 1975, St. Louis & Sulakhe 1976, Fabiato & Fabiato 1978) and it is likely that one or more of these cellular structures is intimately involved in the failure of the contractile response of heart muscle during anoxic and ischemic conditions. The functions of the various organelles of cardiac muscle cells during heart failure is discussed in a detailed review by Dhalla, Das & Sharma 1978.

A detailed investigation of the anoxic interactions which occur between and within different cellular and subcellular structures is thus necessary under various conditions, to explain the state of each one of these structures as reoxygenation commences.

While several investigations have been made of the response of mammalian cardiac muscle to hypoxia and reoxygenation (see Tyberg et al. 1970, Bing et al. 1976), there have been no reports of the interaction of calcium and hydrogen ions during these treatments in either mammals or amphibia.

The present study was therefore performed to examine the role of calcium ions in the contractile response of frog ventricle muscle strips during

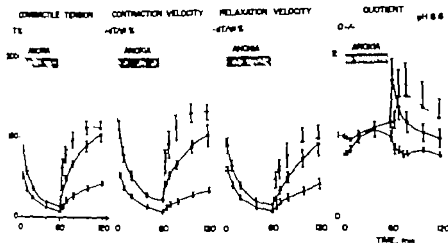


Fig. 2. Mean (±S.E.) of the %-values of the isometric contractile tension, contraction- and relaxation velocities and the quotient of the velocities, during anoxia and reoxygenation at pH 6.6. Series IV expts. with 1.6 mM Ca (▲—▲), series V expts. with 7.0 mM Ca (●—●) and series VI expts. with 1.6 mM Ca during anoxia and 7.0 mM Ca during reoxygenation. Symbols as defined in Table 1. The results of expts. of series VI are only graphed during reoxygenation, as the course of events during anoxia closely followed the results obtained in the series IV expts.

reoxygenation are of particular interest and will be dealt with separately as follows.

A. *Anoxic decay of contractile tension.* The remaining contractile tension after 60 min of anoxia in experiments run at pH 7.6 is significantly less at 1.6 mM Ca^{2+} than at 7.0 mM Ca (T = 37±2% T = 63±4%, $P<0.001$). Also the pre-anoxic values for preparations in low calcium are 100% and for those in high calcium are 144±6%. This then would indicate that the high calcium muscle has lost more contractile tension during the anoxic period than has the low calcium muscle ($P<0.01$).

At pH 6.6 the contractile tension declines to 71% of its initial value. Anoxia results in a further reduction of the contraction strength which at this pH also gives a resultant contractile tension which is significantly less when the calcium concentration is 1.6 mM than when it is 7.0 mM (T = 8±1%, T = 14±1%, $P<0.01$). As pre-anoxic tension values are 71±3% (1.6 mM Ca) and 115±8% (7.0 mM Ca) respectively, contractile tension loss is again greater at the higher calcium concentration than at the lower concentration ($P<0.01$).

On the other hand, although significant differences exist between the resultant tension values left after 60 min of anoxia at pH 7.6 and 6.6 ($P<0.001$) the percentage tension lost during anoxia is identical for experiments using the same calcium concentration ($P>0.05$). During low calcium concentration (1.6 mM) the maximum contraction and relaxation velocities show almost the same percentage changes as does the function of contractile tension at both high (7.6) and low (6.6) pH.

However, elevation of calcium concentrations from 1.6 to 7.0 mM causes a dramatic difference between the contraction and relaxation velocities. The contraction velocity at both pH 7.6 and 6.6 is about twice the rate with 7.0 mM calcium as it is with 1.6 mM calcium. The relaxation velocity on the other hand increases only in proportion to the increase in contractile tension (from 100% to 144% at pH 7.6 and from 71% to 115% at pH 6.6) after addition of 7.0 mM Ca (cf. Figs. 1 and 2). These differences are also observed during anoxia. The quotient of the contraction and relaxation velocities is 0.79±0.02 at both high and low pH. It increases to 1.20±0.07 at pH 7.6 and to 1.08±0.03 at pH 6.6 after calcium elevation from 1.6 to 7.0 mM. The velocity quotients are stable during anoxia at pH 7.6, but increase towards the end of the nitrogen treatment at pH 6.6.

B. *Reoxygenation recovery of contractile tension.* The ventricular muscle strips exposed to anoxia at normal pH recover upon reoxygenation to pre-anoxic contractile tension values within 20 min, independent of the calcium concentration. Cardiac muscle preparations kept at pH 6.6 at low

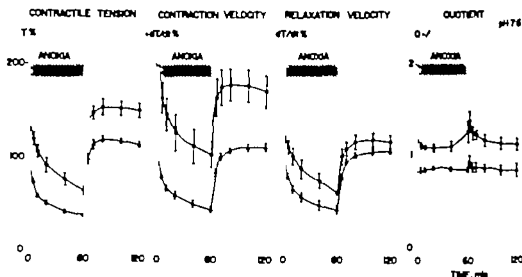


Fig 1 Mean (±S E) of the %-values of the isometric contractile tension contraction- and relaxation velocities and the quotient of these velocities during anoxia and reoxygenation at pH 7 6 Series I expts. with 1 6 mM Ca (▲—▲) and series II expts with 7 0 mM Ca (●—●) are as defined in Table 1

reoxygenation following 1 h of nitrogen anoxia under both acid and normal physiological conditions

MATERIALS AND METHODS

Specimens of the frog *Rana temporaria*, with a body weight of between 10–18 g were used in this study They were maintained in fresh water at 8–12°C The animals were decapitated and despinalised and the heart ventricle dissected ventrally from within the pericardium and separated from the atria. The ventricle was immediately put into a cold (12°C) aerated balanced salt solution of the following composition. Na 16..5 K 5 1 Ca 1 6, Mg 0 9 Cl^- 132.3 SO 0 9 H PO 2.8 HCO_3 35 7 mM

Two ventricular strips with an average cross-sectional area of 1 15±0.04 mm were cut from the apex to the base of each ventricle and mounted in organ baths containing 50 ml of the above balanced salt solution The solution was kept at 12 °C and aerated with a gas mixture of 97% O_2 and 3% CO_2 to give a pH of 7 6

The upper apical end of the strip was connected to a Grass FT03 isometric transducer The lower basal end was attached to a fixed muscle holder The isometric tension and the velocity of its development expressed as the first derivative of the tension function were registered on a Grass Polygraph model 7 The muscle strips were paced at a frequency of 12 stimulations/min of 5 ms duration and 200% of threshold voltage Stimulation was performed with platinum thread electrodes The muscle strips were stretched and left to beat isometrically at their maximum contractile tension of 10.536±0 746 mN/mm² which is described as L_{max} value

A number of experimental series were performed varying both the Ph and Ca^{++} concentration as set out in Table 1 Following a stabilization period of 30–60 min each preparation was aerated with a mixture of 97% N +3% CO_2 for 60 min to produce anoxia. This was followed immediately by 60 min of reoxygenation under the 97% O +3% CO_2 mixture

The calcium concentration was raised by adding 0.1 ml of a 1.84 M $CaCl_2$ solution to the organ bath allowing the muscle to stabilize before commencement of anoxia

The muscle strip exposed to pH 6 6 was also left to stabilize at the new pH before the start of anoxia. Acidification was achieved by slowly adding approximately ml of 12 M HCl to the bath while continuously reading the pH Contractile tension and both contraction (+dT/dt) and relaxation (−dT/dt) velocities were expressed as a % of the stabilized L_{max} value The Student *t*-test was used to determine significant differences

RESULTS

The results for each of the different experimental conditions are graphically presented in Figs 1 and 2 The decay of contractile tension during anoxia and the recovery of contractile tension upo

Table 1 *Ca c ncentration and pH in different experimental series*

Experimental series	No of expts	Anoxia pH	Anoxia Ca (mM)	Recovery pH	Recovery Ca (mM)
I (control)	9	7 6	1 6	7 6	1 6
II	6	7 6	7 0	7 6	7 0
III	3	7 6	1 6	7 6	7 0
IV	9	6 6	1 6	6 6	1 6
V	8	6 6	7 0	6 6	7 0
VI	6	6 6	1 6	6 6	7 0

However, it is not possible to know the extent to which the intra-cellular pH is decreased. According to Ellis & Thomas (1976) the change in intracellular pH of sheep heart Purkinje fibre following a reduction of the external bicarbonate concentration was small and slow. If this is also true for these frog cardiac muscle cells, the fact that the contractile tension recovers more slowly in muscle strips treated in 7.0 mM calcium during anoxia than in preparations supplied with extra calcium only during reoxygenation, implies that the combination of acid conditions and high calcium concentration during anoxia renders the sarcolemma less permeable to calcium ions than at low pH and normal calcium content.

Another explanation might be, however, that the high energy phosphate reserves are reduced much more considerably as a result of the stronger contractions occurring during the anoxic period with high calcium than they are at lower calcium levels. Such a reduction would also increase the glycolytic flux, and hence increase the intracellular hydrogen ion concentration. However, it must be noted that the intracellular acidification and possibly reduced energy content cannot have caused impairment of the contractile mechanism to any large extent as contractile tension of high calcium treated preparations after one hour of anoxia is still significantly higher than the contractile tension seen in the low calcium preparations.

Furthermore, if there is also an intracellular decrease in pH resulting from an increase in extracellular hydrogen ion concentration, the possible interactions between hydrogen and calcium ions at subcellular structures may strongly disturb contraction. The sarcoplasmic reticulum sequesters calcium more actively in an acid environment, provided there is enough energy (Nakamaru & Schwartz 1972, Schwartz et al. 1973). Mitochondria are able to accumulate calcium ions not only at the expense of ATP formation, but also in the absence of other respiratory or ATPase activity, by a diffusion potential which is generated by a hydrogen ion gradient (Selwyn et al. 1970, Lehninger 1974). The myofilaments also exhibit competitive interaction between calcium and hydrogen ions, as submaximal tension development is decreased by lowering the pH (Schädler 1967, Fabiato & Fabiato 1978).

Thus, these subcellular structures can accumulate and/or release calcium ions in different ways, depending on their environment. The faster tension recovery in muscle strips treated with high calcium only during reoxygenation indicates that one or more of these intracellular organelles have modified their mechanisms during anoxic high calcium treatment in a way that renders the myocardial cell less sensitive to calcium ions in spite of identical extracellular conditions.

It is clear however from this study that the weak myocardial recovery of contractile tension upon reoxygenation following one hour of anoxia at pH 6.6 can be significantly increased by addition of calcium ions even in acid conditions. Further work will be necessary to elucidate the exact mechanism by which this occurs.

We are indebted to Inga-Maj Örborn and Ursula Schwarz for their careful calculations, to Birgitta Vallander for preparation of the graphs, to Agneta Gustafsson for typing the manuscript and to John McLean for his valued corrections of the English expression in the text and his critical examination of the manuscript. The work was supported by grants from the Anna Ahrenberg's Foundation, the Adlerbert's Research Foundation, the Lingman Cultural Fund and the Wilhelm and Martina Lundgren Scientific Foundation.

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Table 2 *Means (±S E) of %-values of contractile tension contraction and relaxation velocity after 30 min of reoxygenation in expts of series V and VI*

Experimental series	T	+dT/dt	−dT/dt
V	79±6	78± 7	61± 8
VI	108±9	1 4±13	86±17

calcium concentration do not return to preanoxic tension values when reoxygenated After 60 min recovery only 43±3% of the initial contractile tension remains With 7 0 mM calcium ions recovery is almost complete after 1 h in spite of the low pH

Accordingly there is a more pronounced difference in recovery capability between the high and low calcium muscle preparations at pH 6 6 than at pH 7 6 Even during reoxygenation the changes in contraction and relaxation velocities resemble the changes in contractile tension in experiments with low calcium concentration With 7 0 mM calcium the divergent response of contraction velocity mentioned above is maintained

Although it is difficult to observe any differences between the contraction and relaxation velocity during recovery the velocity quotient does however indicate a more rapid increase in the contraction velocity than in the relaxation velocity during the first 3 min of reoxygenation Within these first minutes of recovery the quotients of the velocities show peak values which are higher at the lower pH with high calcium The quotient peak is more prolonged when 7 0 mM calcium is added at commencement of reoxygenation at pH 6 6 (Exp series VI) This group of muscle strips recover from acidified anoxia significantly faster ($P<0\ 05$) than preparations exposed to 7 0 mM Ca even during the anoxic period (Table ?)

No such difference in recovery-rate occurs in the corresponding experiments at pH 7 6 After 60 minutes reoxygenation however there is no significant difference between the % values of recorded parameters in the experimental series V and VI In all experiments with the same calcium content during both anoxia and reoxygenation the quotient values return to preanoxic levels within 1 h

DISCUSSION

The present investigation has shown responses to hypoxia and reoxygenation for frog cardiac muscle which are similar to those shown for mammalia cardiac muscle by Tyberg et al (1970) and Berg e al (1976) However our results provide further insight into the possible mechanisms involved i these responses

Acid conditions have been shown to suppres contractile tension development in frog ventricula isometric muscle strips during recovery from period of anoxia (Poupa & Gesser 1975) Pool et a (1966) showed that the reduction in high energ stores during hypoxia is not the primary limitatic to contractile force development and conclude that depression of myocardial function wa initiate before a decrease in ATP content The presen work also clearly shows that contractile tensio suppression under acid conditions cannot simply b due to a lack of metabolic energy as an increase calcium concentration can induce rapid recover during reoxygenation The poor recovery unde acid conditions following anoxia must therefore b explained by other interactions involving calciu ions As calcium ions activate myofilaments (Kat 1970) and as the negative effect of hydrogen ion on tension development can partly be reversed b increasing the extracellular calcium ion concen tration it seems likely that the depressant action c hydrogen ions on the recovery of contractile ten sion is caused by interactions with calcium bindi and/or releasing structures in the myocardial cell

The first barrier to calcium ion penetration is th sarcolemma which in cardiac muscle cells is a ver important source of calcium ions Unlike skeleta muscle cells the myofilaments of cardiac muscl cells cannot be completely activated by the releas of intracellular calcium alone Thus the myocar dium is highly dependent on an adequate calciu influx through the sarcolemma which either by it self or by its induction of the release of calciu from the sarcoplasmic reticulum (Fabiato & Fabiat 1978) can bring about filament sliding It has als been reported by Kohlhardt et al (1976) that th slow inward current observed during the action po tential is diminished by a decrea e in pH and both St Louis & Sulakhe (1976) and Williamson et al (1975) have shown that the calcium binding affinit of the sarcolemma i inhibited at pH 6 6 compare to pH 7 4

Hence as the cardiac muscle cells in the presen expts are exposed to hydrogen ions in the bathing solution the transport of calcium across the sar colemma is likely to be suppressed

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Mechanism of action of pentobarbital on the contractile system of isolated frog muscle fibres

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The effects of pentobarbital-sodium were studied on single muscle fibres of frog skeletal muscle. I concentration of 0.5 mM pentobarbital potentiated the twitch response without significantly affecting the tetanus amplitude The increase in twitch amplitude was accompanied by marked increase in the rate of force development. The relaxation phase of both twitch and tetanus was prolonged These mechanical changes were associated with an increased duration of the action potential. Pentobarbital also increased the amplitude and the rate of force development during the twitch after suppression by dantrolene The S-shaped curve relating peak contracture tension and log caffeine concentration was shifted to the left by pentobarbital. It is suggested that pentobarbital affects the contractile activity of the muscle fibre (1) by increasing the rate of release of calcium from its storage sites, (2) by prolonging the duration of the action potential and (3) by inhibiting calcium resequestration by the sarcoplasmic reticulum (SR)

Key words. Pentobarbital skeletal muscle single muscle fibre excitation–contraction coupling, twitch potentiation, action potential

Barbiturates are known to have direct effect on muscle contraction. Pentobarbital for example has been shown to potentiate the twitch response of mammalian as well as amphibian muscles (Quilliam 1955a, Gjone 1956, Foulks et al. 1973 Holmberg & Waldeck 1979) The stimulatory effect of barbiturates on the twitch response of muscle has been attributed to an increased duration of the action potential (Quilliam 1955*b* Gjone 1956 Foulks et al 1973, although it has been suggested that they may augment the twitch by interfering with the relaxation mechanism (Gjone 1956) This latter assumption is supported by the findings that barbiturates inhibit calcium uptake by the sarcoplasmic reticulum (SR) isolated from heart (Briggs et al 1966)

The present study was undertaken as an attempt to obtain further information concerning the mode of action of barbiturates on skeletal muscle Evidence will be presented to show that pentobarbital greatly potentiates the twitch response and also prolongs relaxation in isolated frog muscle fibres The twitch potentiation may be attributed to an increased duration of the action potential It is suggested that the slowing of the relaxation is due to an inhibitory effect of pentobarbital on calcium resequestration by the SR.

METHODS

Effects of pentobarbital were studied on muscle fibres isolated from the ventral head of the semitendinosus muscle of *Rana temporaria*. The methods used for mounting, stimulation, temperature control and for production of caffeine contracture are described elsewhere (Khan & Edman 1979)

Recording of membrane potential. Resting and action membrane potentials were recorded in the presence and absence of pentobarbital (0 5 mM) using conventional glass capillary electrodes (Khan & Edman 1979)

In these experiments the bath temperature varied between 2.8 and 3 3°C but was maintained constant to within ±0.2°C throughout any particular experiment, even during exchange of solutions.

Solutions A Ringer solution of the following composition was used (mM): NaCl 115.5 KCl 2.0 $CaCl_2$ 1.8 Na-phosphate buffer 2.0 pH 7.0

Pentobarbital-sodium (0.05–1.0 mM), barbitone-sodium (1 0 mM) and dantrolene-sodium (9.0 μM) were dissolved in Ringer solution and care was taken to adjust the pH to 7.15

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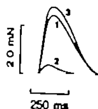


Fig. 2 Effects of pentobarbital on isometric twitch of frog single muscle fibre pretreated with dantrolene. (1) Control, normal Ringer solution. (2) In the presence of dantrolene (9.0 μM). (3) In the presence of dantrolene (9.0 μM) and pentobarbital (0.2 mM).

barbital, the tetanic force however was slightly depressed. Similar to the situation in the twitch response, the time to half decay of tension after the last stimulus of the tetanus response was also prolonged.

The effects produced by pentobarbital were, to a large extent, reversible by repeated washing of the fibre with Ringer solution. An increase of the pentobarbital to 2.0 mM rendered the fibre inexcitable to electrical stimulation.

Dantrolene, a powerful muscle relaxant, is known to suppress the twitch response in a single muscle fibre (Hainaut & Desmedt 1974, Desmedt & Hainaut 1977, Edman 1979). The effects of pentobarbital were also studied in the presence of dantrolene (9.0 μM). As illustrated in Fig. 2, pentobarbital (0.2 mM) potentiated the dantrolene suppressed twitch with a marked increase in the rate of force development.

Contractures were induced by different concentrations of caffeine in the presence and absence of pentobarbital (0.5 mM). The peak tension developed as plotted against the log concentration of caffeine. As illustrated in Fig. 3, pentobarbital shifted the S-shaped curve relating contracture tension and log caffeine concentration to the left.

Other barbiturates like barbitone with low lipid solubility seem to have little effect on the contractile activity of the muscle fibre. As illustrated in Fig. 4, barbitone in a concentration of 1.0 mM had almost no effect on the twitch response.

Influence of pentobarbital on resting and action membrane potential

Pentobarbital did not cause any significant change of the resting membrane potential. The overshoot and the maximum rate of rise of the action potential were also not changed significantly. Pentobarbital,

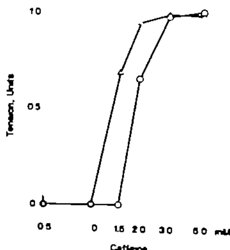


Fig. 3 Effects of pentobarbital on the relation between peak contracture tension and caffeine concentration (log scale) in single muscle fibre. O in the absence of pentobarbital. Δ, in the presence of pentobarbital (0.5 mM). The maximum tension developed by 5 mM caffeine in the absence of pentobarbital was taken as 1.0 unit. Each point represents mean value of 3 different expts.

however reduced the rate of repolarization, causing an increased duration of the action potential (Fig. 5 and Table). These results are consistent with earlier findings in studies on whole muscle preparations (Quilliam 1955*b*, Thesleff 1956, Foulks et al. 1973).

DISCUSSION

The results presented here show that pentobarbital potentiates the isometric twitch amplitude of the single muscle fibre and increases the duration of the action potential. These findings are in agreement with results obtained previously in studies of mammalian and amphibian whole muscles (Quilliam

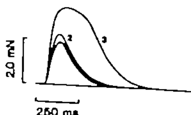


Fig. 4 Comparison of pentobarbital and barbitone effects on isometric twitch of single muscle fibre. (1) Control normal Ringer solution. (2) In the presence of 1.0 mM barbitone. (3) In the presence of 0.5 mM pentobarbital.

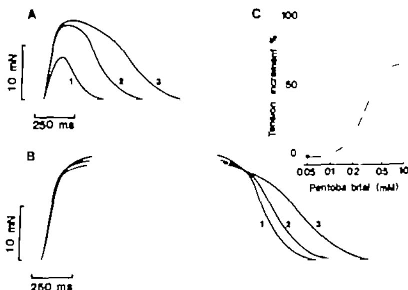


Fig 1 Effects of pentobarbital-sodium on isometric twitch (A) and tetanus (B) in frog single muscle fibre (1) Control normal Ringer solution (2) 0.5 mM pentobarbital. (3) 1 0 mM pentobarbital Stimulus markers below the base line Concentration-response curve (C) Ordinate increase in twitch tension in per cent of control (no pentobarbital). Abscissa pentobarbital concentration (log scale) Each point represents mean value of 3 expts

All chemicals used were of analytical grade and were dissolved in double distilled water

Pentobarbital-sodium and barbitone-sodium were obtained from B D H Chemicals, Poole England Dantrolene-sodium was from Eaton Chemicals Norwich N Y USA

RESULTS

The effects of pentobarbital were studied on twitch and tetanus responses of single muscle fibres It can be seen in Fig 1A that pentobarbital (0 5 and 1 0 mM) greatly potentiated the twitch amplitude and increased the rate of development of tension According to the concentration response curve (Fig 1C) maximum potentiation of the twitch was obtained with 1 0 mM pentobarbital The increased amplitude of the twitch was accompanied by a slight increase in the time to peak tension and a marked prolongation of the relaxation phase (Fig 1A and Table 1) The magnitude of the twitch potentiation was inversely related to the twitch/tetanus ratio recorded in normal Ringer solution When the effect of pentobarbital was maximal (1 0 mM) the amplitude of the twitch reached about 90% of the tetanic force

Pentobarbital (0.5 mM) caused no significant change in the amplitude of the tetanus response (Fig. 1B) At a bath concentration of 1 0 mM pento

Table 1 *Effects of pentobarbital (0 5 mM) on the twitch parameters in frog single muscle fibres*
Each value represents the mean of 4–6 twitch responses

	Normal Ringer		0.5 mM pentobarbital			
Expt. no	Time for 1/2 peak tension (ms) T_c	Time for 1/2 relaxation (ms) R_c	Time for 1/2 peak tension (ms) T	Time for 1/2 relaxation (ms) R_p	T_p/T	R_p/R_c
1	61	96	79	219	1 3	2.3
2	53	114	70	324	1.3	2.8
3	61	88	88	167	1 4	1 9
Grand mean	58	99	79	236	1.3	.3

[illegible] 60–739) (Balzer & Khan 1975 Tada et al 1978). Previous studies suggest that drugs like reserpine, chlorpromazine and prenylamine exert their inhibitory action on SR calcium uptake by binding to the unsaturated fatty acids of the SR lipid phase (Balzer et al 1968). A similar binding of pentobarbital to the lipid phase of the SR might cause a slowing of calcium reuptake and hence be responsible for the prolongation of the relaxation phase.

In conclusion pentobarbital seems to influence the contractile activity of the muscle fibre in 3 different ways

1 By enhancing the *rate* of release of calcium from the storage sites, pentobarbital causes an increased rate of force development and an increased amplitude of the isometric twitch.

2 By prolonging the duration of the action potential the release of calcium occurs over a longer time. This effect will add to factor 1 causing an increased amplitude of the isometric twitch

3 Pentobarbital probably inhibits the calcium resequestration by the SR leading to a reduced rate of relaxation of the twitch and tetanus response

This work was supported by grants to Professor K. A. P Edman from the Swedish Medical Research Council (Project No 14X 184) and from The Muscular Dystrophy Association of America and personal grants to the author from The Medical Faculty University of Lund

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Table 2 *Effects of pentobarbital (0.5 mM) on resting and action membrane potentials of frog muscle fibres (mean ± S E)*

Before recordings were made the fibre bundles were exposed to pentobarbital for 15–20 min. Number of fibres for each measurement given within brackets Student's t-test P<0 001

	Resting potential (mV)	Action potential Overshoot (mV)	Maximum rate of rise (V/s)	Maximum rate of decay (V/s)	Duration at −5 mV level (ms)
Control	86 9± 7 (12)	40 0±_8 (12)	64_±5 1 (12)	77 7±1 0 (12)	4 8±0 (12)
Pentobarbital	84 4± 3 (12)	37 4±2 9 (12)	63 ±6 4 (1)	18.5±1.5 (12)	6.2±0 4 (12)

1955*b* Gjone 1956 Thesleff 1956 Foulks et al 1973) As the action potential may be assumed to govern the release of activator calcium (Sandow et al 1965 Edman et al 1966 Taylor et al 1972) its prolongation would cause an increased release of calcium from the storage sites However if this were the only factor causing twitch potentiation then there would be no change of the initial rate of rise of tension The fact that the rate of force development is markedly steeper in the presence of pentobarbital suggests that the *rate* of calcium release is increased not merely the duration of the release Further evidence in support of this idea is provided by the fact that pentobarbital counteracts the effects of dantrolene The latter agent inhibits the depolarization induced release of calcium from the storage sites (Hainaut & Desmedt 1974 Desmedt & Hainaut 1977) leading to a marked decrease of both the rate of rise and amplitude of the twitch (Hainaut & Desmedt 1974 Desmedt & Hainaut 1977 Edman 1979) Pentobarbital restored the speed of force development in the presence of dantrolene suggesting that the rate of release of calcium was indeed increased

On the basis of studies carried out on muscle from the rat diaphragm Gjone (1956) suggested that barbiturates affect muscle contraction by inhibiting the relaxation process It is now well established that relaxation in skeletal muscle is achieved when calcium is resequestrated by the sarcoplasmic reticulum (SR) An inhibitory action of pentobarbital on the calcium pump would therefore prolong the relaxation phase of the twitch as well as of the tetanus response as was actually observed in the present experiments

If pentobarbital inhibits the reuptake of calcium by SR this would tend to raise the calcium concentration in the myofibrillar space during calcium release by caffeine As caffeine is assumed to induce contractures by enhancing the release of calcium from the storage sites (Endo 1975) this would be expected to lower the concentration of caffeine required to induce contracture In accordance with this prediction pentobarbital was found to shift the S-shaped curve relating peak contracture tension and log caffeine concentration to the left This suggests that pentobarbital like diethyl stilboestrol may have an inhibitory action on the SR calcium resequestration Such an effect on calcium pump of the SR may also contribute to the twitch potentiation as suggested previously (Khan 1979)

It appears likely that pentobarbital being very lipid soluble readily penetrates the cell membrane to have access to intracellular structures like SR. The SR membranes are rich in phospholipid contents and have a high percentage of phosphatidyl-

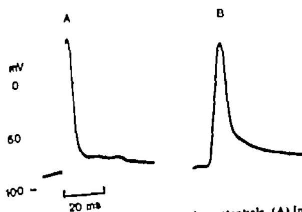


Fig 5 Intracellular recordings of action potentials (A) In normal Ringer solution (B) In the presence of 0.5 mM pentobarbital The recordings in A and B are from different muscle fibres and slightly retouched for greater clarity

cline (60–73 %) (Balzer & Khan 1975, Tada et al 1978). Previous studies suggest that drugs like reserpine, chlorpromazine and prenylamine exert their inhibitory action on SR calcium uptake by binding to the unsaturated fatty acids of the SR lipid phase (Balzer et al 1968). A similar binding of pentobarbital to the lipid phase of the SR might cause a slowing of calcium reuptake and hence be responsible for the prolongation of the relaxation phase

In conclusion pentobarbital seems to influence the contractile activity of the muscle fibre in 3 different ways:

1 By enhancing the *rate* of release of calcium from the storage sites, pentobarbital causes an increased rate of force development and an increased amplitude of the isometric twitch

2 By prolonging the duration of the action potential the release of calcium occurs over a longer time. This effect will add to factor 1 causing an increased amplitude of the isometric twitch.

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Effect of isotonic volume expansion on glomerular filtration rate and renal hemodynamics in the developing rat kidney

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ELINDER, G APERIA, A HERIN P & KÄLLSKOG Ö Effect of volume expansion on glomerular filtration rate and renal hemodynamics in the developing rat kidney Acta Physiol Scand 1980, 108 411–417 Received 20 July 1979 ISSN 0001-6772. Department of Pediatrics, Karolinska Institute St. Görans Children's Hospital Stockholm and Institute of Physiology and Medical Biophysics, University of Uppsala, Sweden.

Young rats (20–24 days) and adult rats (40–45 days) were studied during hydropenia and volume expansion with regard to glomerular filtration rate (GFR) and the determinants of GFR. During hydropenia, GFR and renal blood flow (RBF) were significantly lower in younger than in adult rats both in absolute terms and when related to bodyweight. Equivalent degrees of volume expansion (6% of b wt.) resulted in much more pronounced increase in GFR and RBF in younger than in older rats This suggests that the high renal vascular resistance in hydropenic young rats is primarily due to vasoconstriction. The relationship between the filtration rate of superficial nephrons and the total GFR was the same in hydropenic and volume expanded rats in both age groups. The tubular stop flow pressure the calculated hydrostatic glomerular capillary pressure and ultrafiltration pressure in the afferent part of the glomerular capillaries was slightly lower in hydropenic young rats than in hydropenic adult rats. The pressures did not rise after volume expansion It is concluded that the marked increase in GFR in volume expanded young rats is mainly due to increased renal plasma flow

Key words Renal functional development, intrarenal vascular resistance renal blood flow glomerular filtration pressure, tubular pressure isotonic volume expansion

During early stages of postnatal development it is well documented in man (Aperia et al. 1972), dog (Oelmann & Reuter 1974) lamb (Aperia et al. 1975) and rat (Bengele & Solomon 1973) that the natriuretic response to volume expansion is blunted. This effect has often been ascribed to the low glomerular filtration rate (GFR) of the neonate. It is somewhat surprising, however that the GFR is considerably higher in well hydrated neonatal infants than in hydropenic infants (Broberger 1973 Brodehl et al 1972, Leake et al 1976) This indicates that infants have a reduced natriuretic response to volume expansion despite a large increase in the filtered load of Na. If this is correct, volume expansion in infancy should result in an increased GFR, increased filtered load of Na and increased tubular reabsorption of Na. This would probably lead to an increased energy demand and therefore an increased need for oxygen by the kidney

The aim of the present study was to better elucidate the effects of isotonic volume expansion on GFR and determinants of GFR of the infant rat kidney Hydropenic and volume expanded 20 to 24-day-old rats were compared to hydropenic and volume expanded 40 to 45-day-old rats with regard to renal blood flow GFR single nephron filtration rate and glomerular capillary pressure

In the adult kidney isotonic volume expansion causes an increase in renal blood flow and GFR (Barrat et al 1973 Brenner et al. 1972, Stein et al. 1972, Wallin et al 1971 Wallin et al 1972). The increase in GFR appears to be related to the amount

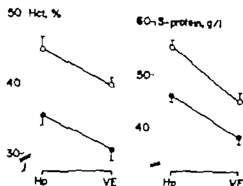


Fig 1 Hematocrit (Hct) and serum protein concentration (C_A) in hydropenic and 6% volume expanded 20 to ?4-day-old rats (closed circles) and 40 to 45-day-old rats (open circles)

of fluid given (Wallin et al 1972) For this reason special attention was paid to the relationship between the degree of volume expansion on the one hand and renal blood flow and glomerular filtration rate on the other

MATERIAL AND METHODS

Male Sprague Dawley rats aged 20–?4 days mean ± S E (23 3±0.2) and 40–45 days mean ± S E (41 9±0 4) delivered from Anticimex were studied during hydropenia and following volume expansion The body weight averaged 63 1±1.4 (mean ± S E) in the younger group and 1?8 9±7 g (mean ±S E) in the older group During this age period there is a linear relationship between body weight and age and between kidney weight and age (Aperia & Herin 1975) The rats were weaned at 17 days of age and prior to the study all rats were given ordinary laboratory diet and water ad libitum.

Most studies have been performed during hydropenia and after isotonic volume expansion corresponding to 6% of b wt ($VE_{6\%}$) Hence the rats were divided into four groups

1*a* ?0 to 24-day-old rats studied during hydropenia
1*b* 20 to ?4-day-old rats studied during volume expansion (6%)
2*a* 40 to 45-day-old rats studied during hydropenia
2*b* 40 to ?4-day-old rats studied during volume expansion (6%)

The following parameters of renal function were determined renal blood flow (RBF) glomerular filtration rate (GFR) single nephron glomerular filtration rate (SNGFR) tubular free flow pressure (P P tubular stop flow pressure (SFP) and pressure in the efferent arteriole ("welling point) (P) The condition of young rats often deteriorates if the duration of the experiment exceeds ? h The studies were therefore performed either during hydropenia or volume expansion In addition different rats were used for RBF and micropuncture studies The preparation of the animals however was the same in most respects in all of the hydropenic and volume expansion studies

For this reason animals in each group had the same average arterial pressure and hematocrit regardless of whether they were used for renal blood flow or for micropuncture studies The hematocrit was lower in younger than in older rats but it was higher in the 6% expanded older rats than in the hydropenic young rats Consequently another group of 40 to 45-day-old rats 2c was included These rats were volume expanded until their hematocrit levels fell within the same range as that observed in the younger rats Their isotonic volume expansion corresponded to 10% of body weight ($VE_{10\%}$) In

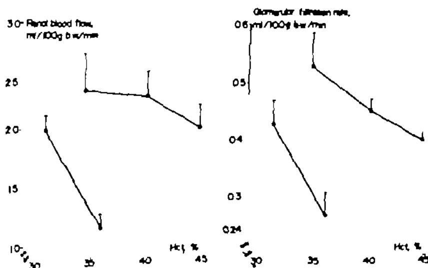


Fig 2 Relationship between hematocrit (Hct) and renal blood flow (RBF) and between hematocrit and glomerular filtration rate (GFR) in ?0 to ?4-day-old and 40 to 45-day-old rats Symbols as in Fig. 1

Table I. *Total renal blood flow (RBF) and glomerular filtration rate (GFR) in hydropenic and volume expanded 20 to 24 and 40 to 45-day-old rat*

Values are mean ±1 S.E. with number of rats per group in parentheses

	RBF (ml/min)	RBF (ml/100 g b.wt./min)	GFR (ml/min)	GFR (ml/100 g b.wt./min)
Ia 20-24 d (Hp)	0.72±0.09 (6)	1.13±0.14 (=6)	0.17±0.03 (=6)	0.27±0.04 (=6)
Ib 20-24 d (VE₆%)	1.30±0.01 (8)	1.99±0.11 (=8)	0.27±0.03 (=5)	0.43±0.04 (=5)
40-45 d (Hp)	3.42±0.34 (=6)	2.01±0.1 (=6)	0.73 0.05 (=6)	0.41±0.03 (6)
40-45 d (VE₆%)	3.95+0.33 (7)	2.29+0.19 (=7)	0.81±0.04 (=5)	0.45±0.02 (=5)
40-45 d (VE₁₀%)	4.14±0.45 (6)	2.42±0.26 (=6)	0.94 0.08 (=8)	0.53±0.05 (=8)
Ia-Ib	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.001$
		n.s.	n.s.	n.s.
	$P<0.001$	$P<0.002$	$P<0.001$	$P<0.02$
	$P<0.001$	n.s.	$P<0.001$	n.s.
		n.s.	n.s.	n.s.
	$P<0.02$	$P<0.02$	$P<0.001$	$P<0.001$

... only the total RBF and the total GFR were measured.

Micropuncture procedures. The rats were prepared for micropuncture in the same manner described elsewhere from this laboratory (Aperia & Herin 1975). Micropuncture was performed under a Leitz stereo microscope at a magnification of 64 and with the aid of one or two Leitz micromanipulators. The SNGFR was determined in early segments of the proximal tubule with sharpened glass capillary (Schnermann et al. 1969). The tip diameter varied between 6 and 9 µm depending on the size of the animal. The tubular fluid was collected spontaneously proximal to a blocking oil drop for time intervals varying between 60 and 200 s. The longer sampling time was used in the younger animals. Approximately 4 collections were made in each animal and the SNGFR was calculated as the mean of all values obtained. The GFR was obtained simultaneously by collecting urine from the ureter via a polyethylene catheter that was advanced almost to the entrance of the pelvis. In some experiments it was suspected that the catheter caused partial obstruction. In these cases the catheter was immediately removed to allow free flow of urine. In those rats only SNGFR could be estimated. Arterial blood samples were taken from the femoral artery catheter. The concentration of inulin in blood and in urine samples was analyzed with the anthrone method (Führ et al. 1955). The hydrostatic pressures were measured with a servo-controlled counter pressure device based on the principles outlined by Wiederhielm et al. (1964) and modified by Intaglietta et al. (1970). In all animals each pressure parameter was determined at least 4 times during hydropenia and during volume expansion. The average value for each animal was then calculated.

Determination of oncotic pressure. Plasma protein concentration (C_p) was determined from arterial blood samples with the Biuret method (Kingsley 1939). The albumin–globulin quotient was obtained by plasma electrophoresis. Since the quotient averaged 1.1 it was thought to be justified to use the equation given by Landis & Pappenheimer (1963) for calculation of the oncotic pressure in arterial blood (2.1C +0.16C² +0.009C³; Eq. 4).

Determination of blood flow. Microspheres (⁵¹Cr 3M Co. St. Paul, Minnesota) measuring 15 µm in diameter (mean ±1 S.D. (14.6±0.8)) were used to quantify the renal blood flow. The method used in our laboratory earlier has been described elsewhere (Aperia & Herin 1975). Absolute values for blood flow were obtained by the reference

100-mm Hg /min ml /100g bw

80-

60-

Hp VE

Fig. 3. Total renal vascular resistance (RVR) in hydropenic and 6% volume expanded 20 to 24 and 40 to 45-day-old rats. Symbols as in Fig. 1.

Table 2 *Nephron filtration rate intrarenal pressure and arterial and venous blood pressures in hydr and volume expanded 20 to 24 and 40 to 45-day-old rats*

		SNGFR (nl/min/ 100 g b wt)	P_{GC} (mmHg)	SFP (mmHg)	Π_{AA} (mmHg)	P_T (mmHg)	P (mmHg)
1a	20–24 d (Hp)	5 99±0 71 (n=8)	45 0±0 9 (n=5)	31 3±0.5 (n=8)	14 1±0.5 (n=5)	14.5±0.8 (n=8)	13 7±1.3 (n 6)
1b	20– 4 d ($VE_{4\%}$)	11 90±1 18 (n=8)	38 6±4 9 (n=5)	28.3±? 0 (n=5)	10.3±1.3 (n=5)	14 6±3 4 (n=5)	12.7±4 7 (n 5)
a	40–45 d (Hp)	10 5 ±0 54 (n=8)	51 8±1 7 (n=5)	33 3±1 6 (n=6)	18 1±0 8 (n=5)	1 4±0 6 (n=6)	12.9±0.3 (n 6)
2b	40–45 d ($VE_{4\%}$)	12 79±0 40 (n=5)	50 5±3 6 (n=6)	36 9±1.5 (n=5)	13 6±1 3 (n=5)	13.–±1.5 (n=6)	1–7±–0 (n 6)
t Test							
1a–1b		$P<0\ 001$	$P<0\ 05$	$P<0\ 02$	$P<0\ 01$	n s	n s.
2a– b		n s	n s	n s.	$P<0\ 001$	n s.	n s
1a–2a		$P<0\ 01$	$P<0\ 01$	n s	$P<0\ 001$	$P<0.02$	n.s.
1b–2b		n s	$P<0\ 002$	$P<0\ 01$	$P<0\ 01$	n s.	n s.

Values are mean ±1 S E. with number of rats per group in parentheses.

sample method" (Aperia & Herin 1975) Nucleotide counting was performed in a Packard Auto-Gamma Spectrometer

Arterio- enous pressure Hematocrit The arterial blood pressure (P_A) was determined by connecting the arterial catheter to a Hewlett Packard pressure recorder Renal venous pressure (P) was measured following puncture of the renal vein with a sharp needle connected to the pressure recorder The arterial hematocrit (Hct) was determined by centrifuging blood in heparinized glass capillaries at 10 000 rpm for 5 min.

Abbreviations

C	Arterial plasma protein concentration
P_{GC}	Hydrostatic pressure in glomerular capillaries
P_T	Hydrostatic pressure in tubules during free flow
$P_{UF\,AA}$	Ultrafiltration pressure in afferent parts of glomerular capillaries
SFP	Stop flow pressure
Π_{AA}	Oncotic pressure in afferent arterioles
RVR	Renal vascular resistance

Eq ations

1 $P_{\ \ AA} = SFP - P$

$P_{GC} = SFP + \Pi_{AA}$

3 $RVR = (P - P_v)/RBF$

4 $\Pi_{AA} = 2.1C + 0\ 16C + 0\ 009C$

The Student s t-test was used for data analysis Probability values of les than 0 05 were regarded as significant

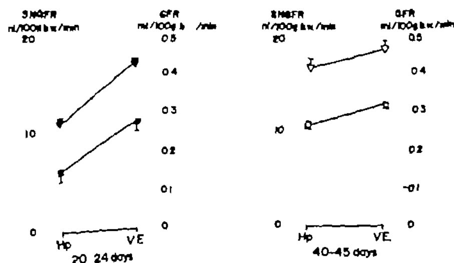


Fig 4 Changes in SNGFR (squares) and GFR (triangles) induced by olume expansion in 20 to 4-day-old rats (filled symbol) and 40 to 45-d y-old rat (unfilled symbols)

Hg)	P (mmHg)	P_{AA} (mmHg)
±13 7	3 5±0 4 (5)	16.8±1.0 (8)
±19 9	2 6±0 7 (n 5)	13.7±5 6 (=5)
±16 6	4 5±0 8 (n 5)	21.3±1 7 (=6)
±13 4 8	5 2±0.7 (n=5)	23 9±3 1 (=6)
	$P<0.001$	s.
		s.
	$P<0.001$	$P<0.05$

ESULTS

he degree of saline expansion was assessed by ermination of the hematocrit (Hct) and the um protein concentration (C_A) (Fig. 1) Although e hematocrit and the serum protein concentration ere consistently lower in younger than in older ts, the infusion of isotonic saline to an amount responding to 6% of b wt. resulted in an equivalent decrease in both parameters in both age oups

Renal blood flow (RBF) and glomerular filtration te (GFR) are shown in Table 1 During hydropenia, both RBF and GFR were higher in older han in younger rats The differences were significant both in absolute terms and when values were lated to body weight

During olume expansion RBF and GFR increased in both age groups With an equivalent degree of volume expansion, however the increase in RBF and GFR were more pronounced in ounger than in older rats When the GFR and RBF ere related to body weight there was no significant fference between 20 to 24-day-old rats and 40 45-day-old $VE_{6\%}$ rats. The relation between hanges in hematocrit and RBF and GFR are shown (Fig 2 Since all groups of rat are included in this ure the hematocrit values for young and old rats erlap It is apparent that the fall in hematocrit duced by olume expansion is associated with sharper rise in renal blood flow and GFR in younger than in older rats

Renal vascular resistance (RVR) was determined (Eq 3) in hydropenic and $VE_{6\%}$ rats of both age groups (Fig 3) During hydropenia, RVR was almost 1.5-fold higher in younger than in older rats $VE_{6\%}$ resulted in a more pronounced fall in RVR in younger (1 7-fold) than in older rats (1 1 fold)

The results of the micropuncture studies are shown in Table 2. In hydropenic rats, the SNGFR in absolute terms as well as in relation to b wt. was significantly lower in younger than in older rats During volume expansion, SNGFR increased more in younger than in older rats It is evident that the age dependent as well as the hydration dependent changes in SNGFR and total GFR parallelled each other (Fig. 4) Table 2 includes the results from the intrarenal pressure recordings in young and adult hydropenic rats which are in complete agreement with previous findings (Allison et al 1972) During hydropenia stop flow pressure (SFP) and hydrostatic pressure in the glomerular capillaries (P_{GC}) as well as the ultrafiltration pressure at the beginning of the glomerular capillaries ($p_{UF,AA}$) were lower in younger than in older rats. In the $VE_{6\%}$ animals, these differences between young and old rats became more pronounced, since $VE_{6\%}$ induced a significant fall both in P_{GC} and SFP in young rats but had no effect on these parameters in older rats The pressure in the efferent arterioles (P) was the same in all groups. The arterial blood pressure was lower in younger than in older hydropenic rats $VE_{6\%}$ had no characteristic effect on arterial blood pressure in either age group

DISCUSSION

The results of the present study show that an equivalent degree of volume expansion (VE) results in a more pronounced increase in RBF and GFR in the immature than in the mature kidney An enhanced effect of VE on GFR in the human neonate has also been observed but it has usually not been discussed (Broberger 1973 Brodehl et al. 1972, Leake et al 1976) The finding that the immature kidney is more sensitive to vasodilatation caused by VE suggests that the immature renal vascular bed is markedly constricted during basal conditions The results of this study therefor provide further evidence for the hypothesis that the low RBF during early postnatal

life is due to physiological rather than to anatomical factors (José et al 1974 Tucker & Blantz 1977)

Although a positive salt and fluid balance results in a relatively larger increase in GFR in younger than in older rats the implications of these findings for the acute control of fluid and electrolyte homeostasis remain uncertain

A VE corresponding to 6% of b wt induced not only a pronounced but also a parallel increase in RBF and GFR in the young animal This provides further insight into some of the factors that govern the GFR and which are of particular importance during late postnatal development Since vasodilatation caused by VE may acutely increase the GFR almost 2 fold and result in GFR values that do not differ from those in the adult animal when related to b wt it is unlikely that the low water permeability of the glomerular capillaries is of importance in lowering the GFR at this developmental stage

The second part of this study was devoted to a more detailed examination of the dynamics of nephron filtration during hydropenia and VE in the developing rat In the superficial nephron accessible to micropuncture the SNGFR increased to a similar extent as the total GFR (Fig 4) This indicates that no redistribution of GFR takes place during the transition from hydropenia to $VE_{6\%}$ The stop flow pressure glomerular hydrostatic pressure and ultrafiltration pressure in the afferent end of the glomerular capillaries were somewhat lower in hydropenic young rats than in hydropenic old rats The difference in P_{GC} between 20 to 24 and 40 to 45-day-old hydropenic rats was however much less pronounced than the previously observed difference in P_{GC} in the neonatal and adult guinea pig (Spitzer & Edelmann 1971) Thus although an increase in the hydrostatic component of the ultrafiltration pressure may well explain the early postnatal rise in GFR in guinea pigs it is probably of minor importance for the late postnatal rise in GFR in rats

It is noteworthy however that while a 6% volume expansion resulted in an almost 2 fold increase in SNGFR it actually decreased the hydrostatic pressure in the glomerular capillaries in young rats Thus by exclusion it can be concluded that glomerular blood flow must be a major determination of the GFR during acute changes in fluid balance in the 20 to 24-day-old rat. It seems likely that renal blood flow exerts its effect on the GFR not only by presenting more plasma fluid/unit time to the filtering capillaries but also by changing the oncotic pressure along the length of the glomerular capillaries It is of interest in this connection that in a previous study from this laboratory it was shown that during postnatal development from 20 to 40 days of age the GFR also increases mainly as a function of RBF (Aperia & Herin 1975)

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Cholinergic vasoconstrictor effects in the rabbit eye
Vasomotor effects of pentobarbital anesthesia

ANDERS BILL and JOHAN STJERNSCHANTZ

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Oculomotor nerve stimulation causes vasoconstriction in the anterior uvea, which is due partly to muscarinic mechanism and partly to non-sympathetic aminergic mechanism. The labelled microsphere method was used to analyze the effect of pentobarbital anesthesia on the resting cholinergic vasomotor tone in the anterior uvea and to determine the relationship between stimulation frequency and vasomotor response. An attempt was made also to ascertain whether the aminergic part of the vasoconstriction is caused by release of 5-hydroxytryptamine or norepinephrine. Induction of pentobarbital anesthesia caused marked vasodilation in the iris and the ciliary processes and subsequent muscarinic blockade had no effect on the blood flow A similar result was obtained in the optic nerve. In the choroid plexus heart muscle, pineal body and coecum, pentobarbital anesthesia caused vasodilation and a subsequent muscarinic blockade caused vasoconstriction. I the brain pentobarbital anesthesia caused marked reduction in the blood flow of the grey matter and moderate reduction in the white matter After a muscarinic blockade there was some increase in the blood flow of the grey matter Stimulation of the oculomotor nerve caused near maximum vasomotor responses at 10–20 Hz; maximum effect on the pupil size was obtained at 40–50 Hz Depletion of 5-hydro ytryptamine with fenfluramine did not prevent the aminergic part of the vasoconstriction and marked vasoconstriction was also observed after pretreatment with reserpine. The results indicate that pentobarbital anesthesia abolishes most of the spontaneous cholinergic vasoconstrictor tone of the anterior uvea and that the aminergic part of the oculomotor nerve induced vasoconstriction is caused by the stimulation of phentolamine-sensitive receptors by mechanism probably not involving release of norepinephrine or 5-hydroxytryptamine.

Key words: Pentobarbital anesthesia, oculomotor nerve stimulation, cholinergic vasoconstriction, blood flow

There is an interesting discrepancy between the in vitro and in vivo responses of the blood vessels to acetylcholine. In vitro acetylcholine usually causes contraction while under in vivo conditions acetylcholine as well as parasympathetic stimulation almost invariably causes vasodilation (see Vanhoutte 1977) An exception to the rule of in vivo vasodilator responses to parasympathetic stimulation was reported recently It was found that intracranial stimulation of the oculomotor nerve caused a vasoconstriction in the iris and the ciliary processes in rabbits (Stjernschantz, Alm & Bill 1976). Part of the vasoconstriction was due to muscarinic mechanism, another part seemed to be due to a non-sympathetic aminergic mechanism involving receptors which could be blocked by phentolamine as well as with methysergide (Bill, Stjernschantz & Alm 1976, Stjernschantz, Alm & Bill 1977). With a complete ganglionic blockade—as well as after phentolamine plus biperiden—oculomotor nerve stimulation tended to cause some vasodilation in the anterior uvea.

A subsequent study demonstrated that in rabbits the cholinergic vasoconstrictor mechanism in the iris is a physiological phenomenon. when a cholinergic blockade was produced in unanesthetized rabbits there was a marked increase in the blood flow of the anterior uvea (Stjernschantz et al. (1977).

Another previous observation was that the blood flow in the control eyes in the experiments with stereotaxic stimulation was already higher than that in unanesthetized animals even before administration of the cholinergic blocking agent (Bill et al. 1976 Stjernschantz et al 1977) This observation indicated that the anesthesia or the other proce

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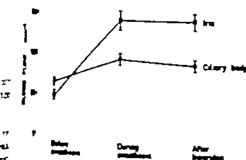


Fig 1

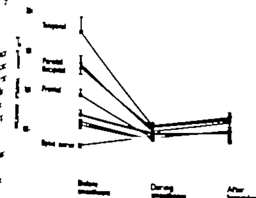


Fig 3

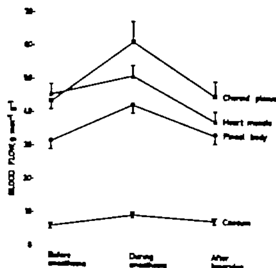


Fig 2

Figs 1–3 Effect of pentobarbital anesthesia and subsequent administration of biperiden on regional blood flow Values shown are mean ±S.E. number of animals = 9 In Fig 3 each region of the brain is represented by one symbol, filled for grey matter unfilled for the white

12.3±1 6 kPa, 4 6±0 4 and 7 31±0 05 respectively Biperiden had no significant effect on the blood pressure or the acid-base balance

Fig 1 shows results for the anterior uvea. Barbiturate anesthesia markedly increased the blood flow in the iris and ciliary body ($P<0.001$). Biperiden then had practically no effect. Fig. 2 shows vasodilating responses to pentobarbital anesthesia in some other tissues Pentobarbital sodium increased the blood flow in the pineal body choroid plexus and large intestine ($P<0.005$) and tended to do so also in the heart muscle ($P<0.05$) After biperiden the flow decreased in all these tissues ($P<0.01$) Fig 3 shows that in the brain there was a marked reduction in the blood flow of the grey matter after the induction of anesthesia. The reduction was highly significant ($P<0.001$) in all parts. After biperiden there was a small but statistically significant ($P<0.01$) increase in the blood flow. In the white matter pentobarbital reduced the blood flow moderately ($P<0.01$) and biperiden had no significant effect. In the optic nerve pentobarbital anesthesia increased the blood flow ($P<0.01$) but biperiden had no statistically significant effect.

Oculomotor nerve stimulation

The effect of oculomotor nerve stimulation on the blood flow in the anterior uvea is presented in Table 1 The reduction in blood flow in the iris was greater than that in the ciliary processes The results were similar in the pigmented and albino rabbits

Fig 4 shows the relationship between the stimulation frequency and the response in blood flow

Table 1 *Blood flow in the anterior uvea (M±S.E.) on the stimulated and control side The flow on the stimulated side was expressed also as percentage of control flow*

Means (±S E) of the percentage values are shown * indicates statistically significant reduction at the $P<0.01$ level and *** at the $P<0.001$ level (=10)

Tissue	Stimulated side (mg min⁻¹)	Control side (mg min⁻¹)	Stimulated side % of control
Iris	39±8	138 25	33±7**
Ciliary processes	57±9	82±10	72±7*
Anterior uvea	95±12	220±33	50±7**

dures involved in stereotaxic stimulation had abolished a vasoconstrictor tone in the anterior uvea A cholinergic blockade under these conditions had only a moderate vasodilating effect in the anterior uvea Previous studies on the effects of barbiturate anesthesia on regional blood flow in rabbits did not include determinations of the blood flow in the eye (Aardal Svanes & Egenberg 1973)

The purpose of the present study was to investigate further the mechanisms involved in the oculomotor nerve control of the uveal blood flow in rabbits The aims were 1) to determine if pentobarbital anesthesia affected vasomotor tone in the uvea and some other tissues 2) to determine the stimulation frequency response relationship of cholinergic vasoconstriction and 3) to try and analyze if depletion of the monoamines affected the response to oculomotor nerve stimulation appreciably

MATERIALS AND METHODS

1 *Effect of pentobarbital anesthesia and cholinergic blockade*

The experiments were performed in albino rabbits of both sexes and weighing 1 8–3.2 kg One day before the actual experiment one femoral artery was cannulated with a polyethylene tubing and the left heart ventricle was cannulated via the left subclavian artery using another tubing Cannulation was performed under pentobarbital anesthesia and the animal recovered within a few hours On the next day the animal was placed in an open box and about 1 2 million radioactively labelled 15 μm microspheres (3M Company St Paul) in 1 ml saline were injected into the left ventricle over 10 s From the start of the injection and for the next 60 s the blood from the cannulated femoral artery was collected The flow rate was about 1 ml/min Anesthesia was then induced with pentobarbital sodium, about 50–60 mg/kg b wt The anesthetic was given slowly i v and at a dose which made it possible to put the animal on its back and pinch the ear without marked reactions After 15 min of anesthesia a new blood flow determination was made using microspheres labelled with another isotope Immediately after the second flow determination cholinergic blockade was accomplished by i.v injection of biperiden A third flow determination was performed 5 min later using microspheres labelled with a third isotope Details of the technique used for flow measurements were described previously (Stjernschantz et al 1976)

2 *Oculomotor nerve stimulation*

Albino as well as pigmented rabbits of either sex and weighing 1 7–4 4 kg were used The animals were anesthetized with pentobarbital sodium initial dose 40–60 mg/kg b wt Additional doses were injected when necessary to maintain anesthesia. All the animals received tubocurarine 0.5–1 mg/kg b wt before stimulation The animals were artificially ventilated and P_{CO_2} P_{O_2} and pH of the arterial blood were checked Both femoral arteries were cannulated one for blood sampling and the other for blood pressure measurement. The animals were thoracotomized and the left heart ventricle was cannulated A mixture of Macrodex (Pharmacia Uppsala) and isotonic saline (1 1) was injected intravenously to increase the blood pressure whenever needed The amount of this mixture did not exceed 40 ml The animals were kept warm by a heating pad

Intracranial stimulation of the oculomotor nerve The intracranial part of the oculomotor nerve was electrically stimulated with a stereotaxic technique (see Stjernschantz 1976 Stjernschantz et al 1976) The nerve was stimulated about 3–4 mm posterior to the place where it leaves the cranial cavity Electrical stimulation was carried out using a bipolar electrode with unidirectional square wave pulses intensity 3–6 V duration 1 ms and frequency ranging from 5–90 Hz mostly being 40–50 Hz. In each experiment a first stimulation to check the position of the electrode was performed with a 40–50 Hz frequency 10–30 min before the first flow determination Only experiments with good miosis immediately reversible after stimulation were accepted The nerve was stimulated for 2 min before the blood flow determination and during it After the experiment the exact position of the electrode was checked

Pharmacologic treatment Cholinergic blockade was induced by biperiden lactate .5–5 mg/kg b wt) The lower dose completely inhibited the response of the sphincter pupille muscle to oculomotor nerve stimulation Fenfluramine chloride was daily administered i m 20–42 h before stimulation in a dose of 10 mg/kg b wt (each day) in order to deplete 5-hydroxytryptamine stores in a selective way At this dosage fenfluramine causes marked depletion of brain serotonin (Duhault & Verdavainne 1967) An additional dose of 5 mg/kg b wt was given i.m. 3–5 h before stimulation Reserpine was administered i.m 20–4 h before stimulation in a dose of 5 mg/kg b wt At this dosage reserpine causes marked depletion of monoamines (Shore Olin & Brodie 1957) which resulted in a low blood pressure An additional dose of 5 mg/kg b wt was given i m 3–5 h before stimulation

RESULTS

Effect of pentobarbital anesthesia and cholinergic blockade

Preliminary experiments indicated that induction of pentobarbital anesthesia had little effect on the blood flow in most tissues caused vasoconstriction in some and dilation in others Some of the tissues in which there seemed to be a change were subjected to a more detailed investigation in a series of nine animals Before anesthesia the mean arterial blood pressure P_{O_2} P_{O_2} and pH in this series were 84±3 mmHg, 13 ±1 5 kPa 4.3±0 4 kPa and 7 38±0 02 respectively After 10–15 min of anesthesia the corresponding values were 74±3 mmHg

tone in the blood vessels of the anterior uvea. A corresponding, almost complete elimination of the activity in the nerve fibres supplying the sphincter muscle cannot be expected. The pupil is wide after pentobarbital anesthesia but it reacts to light and ordinary room light—as used in the present experiments—can thus be expected to cause some activity in the sphincter fibres.

Effects of oculomotor nerve stimulation

The results of the experiments with oculomotor nerve stimulation at different frequencies indicates that the vasomotor effect has a frequency-response relationship very different from that in the sphincter pupillae muscle: near maximum vascular effects are produced already at frequencies between 10–20 Hz. To produce near maximal responses in the sphincter pupillae frequencies around 40–50 Hz had to be added to the spontaneous activity which probably was low.

As mentioned in the introduction vasoconstrictive effects of acetylcholine have been obtained in many in vitro preparations of blood vessels. And such effects have been observed also in artificially perfused organs. If in vitro preparations of arteries are given a tone e.g. by norepinephrine acetylcholine tends to cause dilation which suggests that there are receptors in the smooth muscles for dilation as well as for constriction. Under in vivo conditions there is still another effect of acetylcholine: it tends to reduce the amount of norepinephrine released by the sympathetic nerves, an effect that contributes to making the in vivo response dilatory (Vanhoutte 1977). Usually the in vitro vasoconstrictor effects of acetylcholine as well as the dilative effects are muscarinic but vasoconstrictor effects that could not be blocked by atropine have been reported for the perfused rabbit ovarian artery (Graham & Sani 1971).

In the perfused cat eye acetylcholine as well as stimulation of the ciliary ganglion has been reported to produce effects indicating vasoconstriction in the veins. These effect could be blocked by cholinergic blocking agents as well as by adrenergic alpha-receptor blocking agents and were interpreted to indicate release of norepinephrine from intraocular neuronal terminals (Macri & Cevario 1975).

Previous studies (Stjernschantz & Bill 1979) indicated that in cats and monkeys oculomotor nerve stimulation under in vivo conditions causes vasodilation in the ciliary body and vasoconstriction in the iris. The responses in rabbits were constrictive as in the present experiments. The iris and the ciliary processes have a rich supply of sympathetic nerves, and stimulation of these nerves causes marked vasoconstriction (Stjernschantz et al. 1977). Prerequisites thus seem to exist for one of the mechanism by which acetylcholine could cause vasodilation. And the response in the ciliary body in cats and monkeys may be secondary to inhibition of the norepinephrine release or/and stimulation of vasodilating receptors. But in the iris—and in rabbits also in the ciliary body—the normal response seems to be dominated by stimulation of the constrictive receptors.

Previous studies (Bill et al. 1976, Stjernschantz et al. 1977) indicated that the vasoconstriction in the anterior uvea in rabbits was due partly to a cholinergic and partly to an aminergic mechanism. Vasoconstriction induced by oculomotor nerve stimulation in sympathectomized rabbits ruled out the possibility of a sympathetic co-stimulation (Alm, Stjernschantz & Bill 1976). This has been confirmed further by experiments in which sympathetic blockade has been carried out with bretylium prior to stimulation (unpublished data). It never became clear in the previous studies whether the aminergic mechanism involved receptors only at the target area, or whether there was also a local release of a biogenic amine as suggested originally by Macri & Cevario (1975). From our previous experiments (Stjernschantz et al. 1977) 5-hydroxytryptamine seemed a possible candidate. Since in the present experiments fenfluramine, which is a selective 5-hydroxytryptamine depletor in the brain and in platelets (Picotti et al. 1977) was totally without effects in preventing the aminergic part of the vasoconstriction, this hypothesis received no support. It is not clear at present if there are any 5-hydroxytryptamine nerves in the uvea and whether they would be depleted to an appreciable extent by fenfluramine. Therefore also reserpine was used in depletion experiments, the aim beeing to deplete aminergic nerves containing norepinephrine, 5-hydroxytryptamine or dopamine. The vasoconstrictive effect of stimulation in reserpinized animals differs markedly from the effect in phentolamine treated animals in which stimulation caused vasodilation in the ciliary body (Bill et al. 1976). And the effect of stimulation after biperiden in reserpinized animals was similar to that obtained previously with biperiden only. Thus neither reserpine nor fenfluramine

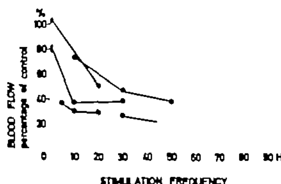


Fig 4 The relationship between stimulation frequency and the iridial blood flow

Marked effects on the blood flow were observed already at 5–10 Hz At these frequences there was little change in the pupil diameter Maximum miosis was obtained only at 40–50 Hz and higher

Rabbits pretreated with fenfluramine showed a distinct vasoconstrictive response to stimulation in spite of a cholinergic blockade (Fig 5) The mean arterial blood pressure after fenfluramine and biperiden before stimulation was 81±4 mmHg and during stimulation 79±? mmHg Pretreatment of the animals with reserpine was in a similar way without effect in blocking the vasoconstrictive response to stimulation (Table ?) Most of the vasoconstriction could be abolished by an additional cholinergic blockade (Table 2) but there remained a probably significant effect in the iris The mean arterial blood pressure after reserpine was 62±3 mmHg and after reserpine and biperiden 62±mmHg

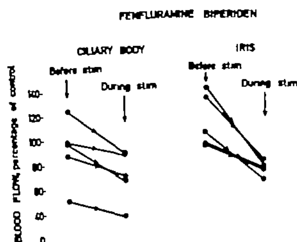


Fig 5 Effect of oculomotor nerve stimulation on the blood flow of the iris and the ciliary processes in rabbits pretreated with fenfluramine and biperiden.

Table ? *Effect of oculomotor nerve stimulation on the blood flow in the anterior uvea in reserpinized animals*

The blood flow is expressed as percentage of the blo[od] flow in the control eye indicates a statistical significa[nce] at the $P<0.05$ level at the $P<0.01$ and at th[e] $P<0.001$ level ($n=10$) (M±S E)

	Iris (%)	Ciliary proc (%)	Anterior uvea (%)
Pretreatment with reserpine	40±7	76±7	57+6**
Pretreatment with reserpine and cholinergic blockade with biperiden	79±8	94±8	85±7

DISCUSSION

Effects of pentobarbital and biperiden

Some of the effects of the pentobarbital anesthe[sia] shown in Fig 1–3 have been observed previousl[y] Goldman & Sapirstein 1973 reported that brai[n] blood flow was reduced in rats as a consequence o[f] pentobarbital anesthesia and that there was a corre[-] lation between preanesthetic values and the reduc[-] tion in flow As in the present experiments, anes[-] thesia tended to reduce blood flow to a relativel[y] uniform level The changes observed in the presen[t] study in the choroid plexus and pineal body ar[e] puzzling The pineal body has been suspected o[f] being involved in stabilizing and deactivating pro[-] cesses in the brain (Romijn 1978) Increased flow may be the result of increased activity in this par[-] ticular part of the brain The observation that afte[r] cholinergic blockade pineal and choroidal plexu[s] blood flow decreased and that at the same tim[e] there was an increased flow in the grey matter raises the question whether there may be a func[-] tional connection between the pineal body and the rest of the brain involving a cholinergic mechanism The reason for increased blood flow in the large in[-] testine after pentobarbital is not clear The reduc[-] tion in flow caused by the cholinergic blockade sug[-] gests that in this tissue there was a cholinergic vaso[-] dilating tone after the induction of anesthesia

The observation that in the anterior uvea pen[-] tobarbital anesthesia caused a very marked increase in blood flow and that a subsequent cholinergic blockade had no effect indicates that the anesthesia virtually abolished the spontaneous cholinergic

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Effects of sprint and endurance-training on capillary circulation in human skeletal muscle

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Several approaches have been used to clarify the effect of endurance-training on structural, biochemical and physiological properties of skeletal muscle A number of investigators (Grimby et al 1967 Clausen et al 1969 Varnauskas et al 1970) have shown that blood flow per kg of working muscle, as measured by ^{133}Xe clearance method at submaximal work level, is slower in trained individuals than in controls. Trained individuals show greater arteriovenous oxygen difference during submaximal exercise, which is presumably due to increased capacity of the trained individuals to extract oxygen from circulation (Saltin et al 1969) We recently showed increased capillary permeability in endurance-trained athletes which is most likely a manifestation of increased capillary surface area (Leinonen et al 1978) This finding is consistent with morphological studies which showed increased muscle capillary density in endurance-trained athletes (Andersen 1975 Brodal et al. 1977 Ingjer 1979) In contrast to endurance-training little is known about the consequences of sprint-training Therefore muscle blood flow (MBF) and capillary diffusion capacity (CDC) were estimated by measuring local clearances of ^{133}Xe and ^{131}I after ischemic exercise in a group of sprinters and the results are compared with the data for distance runners and controls.

Materials and method 6 international-caliber sprinters (3 were in the Finnish Olympic track team) were studied They had been actively training and competing for at least 3 years. Their main training program took the form of short duration daily exercises such as sprinting, interval running and weight lifting In addition, they had a relatively light endurance program of 15–35 km of running per week. The 13 distance runners (3 were Olympic medalists and one double European champion) ran regularly 90–135 km per week. All athletes were studied during their most active competition season. The 10 controls were sedentary adult volunteers. The methods for measurement and calculation of MBF and CDC were in detail described elsewhere (Leinonen et al. 1978) Briefly the clearance rates were recorded with the use of lipophilic ^{133}Xe (50–70 μCi) and hydrophilic ^{131}I (10–70 μCi) dissolved in 0 1 ml of isotonic saline deposited locally with the use of a narrow gauge needle in the anterior tibial muscle made hyperemic with ischemic exercise The rate constants and CDC for ^{131}I were calculated for the initial rapid wash-out slope (0–30s after onset of post-exercise hyperemia) and the secondary "steady-state wash-out slope (30-90 s) of the clearance curve MBF was calculated from the period of maximal hyperemia (30–90 s after onset of post-exercise hyperemia)

Results The results, as well as statistical analyses, are given in Table 1 The sprinters had only a slight, nonsignificant increase in MBF (10%) and in CDC (13%) when compared to controls MBF and CDC were significantly greater in distance runners than in sprinters or controls. The average increment of MBF was 34% and that of CDC nearly 50%

Discussion Being a lipophilic gas ^{133}Xe freely crosses cell membranes the main hindrance to its exchange with blood being the diffusion distances while the exchange of lipid-insoluble substances such as ^{131}I is restrained by capillary membranes The increases of MBF and CDC (particularly during the secondary slope of ^{131}I clearance curve) in distance runners are explained by an increase in the muscle capillary surface area, as discussed earlier (Leinonen et al 1978) This is also supported by recent studies which showed a marked increase in muscle capillary supply when untrained and endurance-trained groups were compared (Andersen 1975 Brodal et al. 1977 Ingjer 1979) or when the same subjects were studied before and after training (Andersen & Henriksson 1977) Furthermore Ingjer (1978) showed that the increase in capillary supply occurs linearly with increasing maximal aerobic power The observed nearly 50% increase

gave results mimicking those of receptor blockade with phentolamine It seems unlikely then that oculomotor nerve stimulation normally results in release of any of the amines mentioned or enhances the effect of some circulating amine Other explanations such as effects of acetylcholine on the aminergic receptors or release of an unconventional transmitter with effects on the vascular aminergic receptors or peculiarities in the acetylcholine receptor structure in the smooth muscle would seem to be more likely alternatives

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Table 1 Average data for controls sprinters and distance runners (mean ± S D)
Figures within parentheses denote number of subjects ns=non significant

Groups	Age (yrs)	Muscle blood flow (ml 100 g min)	Rate constant for [131]I (min) Initial slope	Secondary slope	Capillary diffusion capacity for [131]I (ml 100 g min) Initial slope	Secondary slope
Controls (10)	27 8	62 9± 8 7	0 6_±0 17	0.35±0 07	11 3±3 5	5 6±1.3
Sprinters (6)	23.3	68 9±14 4	0 81±0 18	0.39±0 03	14 5±3 4	6 3±0 6
Distance runners (13)	26 4	84 1±15 1	0 88±0 22	0.51±0 07	15 9±4 4	8.3±1 1
Statistical analysis[a]						
Controls vs sprinters		ns	ns	ns	ns	ns
Controls vs distance runners		$P<0\ 001$	$P<0\ 005$	$P<0\ 001$	$P<0.0_$	$P<0\ 001$
Sprinters vs distance runners		ns	ns	$P<0\ 001$	ns	$P<0\ 001$

[a] Student s *t*-test

of capillary permeability in endurance trained athletes corresponds quite well with the results for maximal oxygen uptake and capillary supply Brodal et al (1977) found that the mean capillary density of skeletal muscle and maximal oxygen uptake were both 40% higher in endurance-trained individuals than in untrained and in Ingjer's (1979) recent study the percentages were 49% and 56% respectively These adaptations to endurance training together with increased oxidative enzyme activity (Varnauskas et al 1970 Andersen & Henriksson 1977) and capillary permeability i e increased transcapillary transport of small molecules all compensate for the lower blood flow measured during dynamic exercise thus securing adequate muscle nutrition

There is however only scarce data dealing with the adaptive response of human muscle to anaerobic sprint training Sprinters and distance runners have distinct muscle fiber compositions the latter having a higher percentage of type I (slow twitch) fibers (Costill et al 1976) This is mostly due to genetic factors and muscle fiber composition probably predetermines athlete s success in either sprint or distance running Jansson and coworkers (1978) however showed that the relative proportion of type I fibers in trained individuals is also decreased by a period of interval training Type I fibers are supplied with the greatest number of capillaries around each fiber also relative to fiber area (Ingjer 1979) This indirect evidence points to the fact that sprint training does not affect muscle capillary supply to a same degree as endurance training This is also reflected in the present observation that MBF and CDC were only slightly increased in sprinters

In conclusion additional studies are needed to describe the changes occurring in muscle due to training especially various stages of sprint and endurance training In addition to structural and histochemical studies the functional changes can easily and noninvasively be studied using the local isotope clearance method

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Acetylcholinesterase positive nerves in the guinea-pig endometrium

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Nerves in the endometrium and nerve terminals to the glands and epithelial linings have been demonstrated by several authors in different mammals e g man, macaque rabbit, guinea-pig and rat with metal impregnation techniques and supra- or intravital methylene blue staining, and also by electron microscopy (vide Hammarström & Sjöstrand 1979). Endometrial nerves have also been shown to be acetylcholinesterase (AChE) positive in man (Coupland 1962, 1969) and in rat (Adham & Schenk 1969). In the guinea-pig endometrium, however no AChE positive nerves have been observed (Jordan 1970, Thorbert et al 1977).

Nevertheless, in a recent study we have obtained physiological and pharmacological evidence of a cholinergic secretory innervation of the guinea-pig endometrium (Hammarström & Sjöstrand 1979). Since the lack of demonstration of endometrial AChE positive nerves in the guinea-pig could be due to technical difficulties with regard to the composition of the endometrial tissue (vide Theobald 1973) we have reinvestigated the matter.

Material and methods The modified Koelle Friedenwald method of Karnovsky & Roots (1964) was used for identifying AChE positive nerves. Virgin guinea-pigs treated with estradiol benzoate (5 μg/kg) and hydroxyprogesterone caproate (150 mg/kg) (Primosiston®) every third day for 6–8 days were stunned and bled. The uterus was carefully dissected out and cut into 5–8 mm pieces. The pieces were either directly frozen (−30°C) or frozen after 1–16 h of fixation in ice-cold formalin (Karnovsky & Roots) followed by 1–4 days in ice-cold gum sucrose -solution (Holt, Hobbiger & Pawan 1960). Some unfixed sections were postfixed in formalin according to El-Badawi & Schenk (1967). Sections (10 μm) were cut in a cryostat. Time in the incubation medium recommended by Karnovsky & Roots was 1–5 h. Iso-OMPA (4 μM) was used to inhibit nonspecific cholinesterase activity. For general staining of the sections eosin or lichtgrün was used.

Result comments and conclusions As expected there were difficulties in demonstrating AChE positive nerves in the endometrium. The most satisfactory results were obtained in sections from pieces fixed for 1 h and incubated in the substrate medium for 3 h. No certain AChE activity was noted in sections from pieces fixed for more than 3 h. Unfixed or postfixed sections exhibited AChE activity but it was difficult to localize properly.

In accordance with earlier studies (Bell 1968, Thorbert et al. 1977) AChE-positive nerves were observed in the parametrial arteries (Fig. 1*a*). However we could also trace such nerves into the myometrial branches (Fig. 1*b*). Also in the myometrium AChE positive fibres were seen (Fig. 1). Furthermore AChE positive nerve trunks were observed in the endometrium not only in the basal part but also in the functionalis (Fig. 1*d*). Moreover they seemed to run free in the stroma and did not accompany arteries. However it was not possible to descern distinct nerve terminals in the lining or glandular epithelium but terminals were noted close beneath (Fig. 1*d* and *e*).

The demonstration of AChE positive nerves in the endometrium supports our findings concerning uterine secretion, i.e. the endometrium has a cholinergic secretomotor innervation (Hammarström & Sjöstrand 1979). Nerves to the guinea-pig endometrial secretory cells have been described by Köstlin (1894) using silver impregnation of osmium tetroxide-potassium bichromate fixed material and by Jacobson & Nieves (1961) using intravital methylene blue staining. The terminals are however difficult to discern why the latter authors were forced to supply the methylene blue solution with hyaluronidase in order to decompose the stroma, for a clear staining and visualization of the terminals. Our study also emphasizes the well-known fact that in AChE histochemistry one must vary the conditions and techniques for each tissue and species in order to obtain suitable results.

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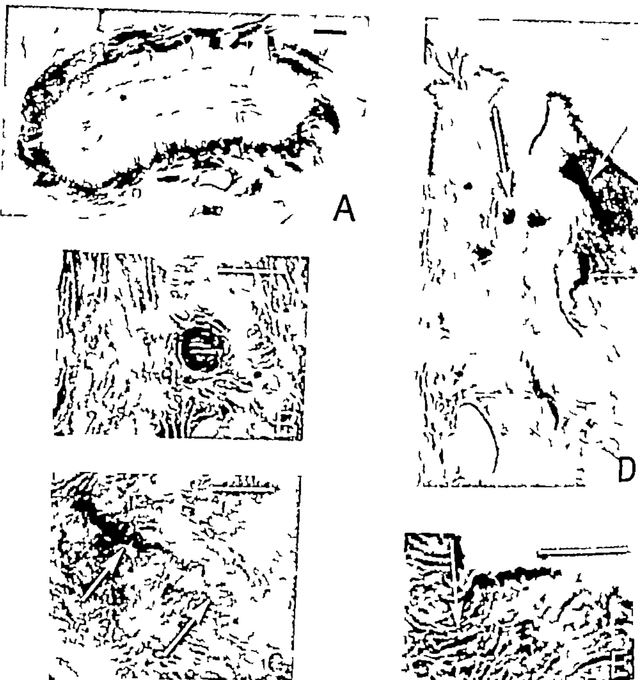


Fig 1 Acetylcholinesterase positive nerves in the guinea pig uterus. Technique of Karnovsky & Roots. General staining with eosin Calibration line 10 μm (A) Intense AChE staining of nerve fibres in a parametrial artery The fibres are mainly localized in the outer border of the media and in the adventitia. (B) Nerve fibres surrounding the smooth muscle in a small myometrial artery (C) Nerve running in the myometrium (not adjacent to larger blood vessels). (D) Nerve trunks and fibres in the endometrium Note the fibres close to glandular epithel um (thin arrows) and larger transversically sectioned trunks below surface epitelium (thick arrows) (E) Small terminal beneath epithelial lining of the endometrium.

Presumably the inability of Jordan (1970) and Thorbert et al (1977) to trace AChE positive nerves within the uterus was simply due to technical matters

We are grateful to Mrs Annika Rosén for the excellent technical assistance in preparing the slides and to Dr Ulrik Kvist and Mr Carl O Löfman for taking the photographs

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Sodium-sensitive receptors involved in water and salt balance

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Studies performed in the goat imply that cerebral receptors regulati g wate i take and the release f asopres i (AVP) have periventricular location and that the activity of these receptors i positi ely correlated to the Na f the cerebrospinal fluid (csf) (cf Andersson 1978) McKinley Denton and Wei inger (1978) suggested that there might be both osmosensiti (as originally proposed by V rney 1947) and sodium sensitive recepto The latte assumption was partly based on the observation that short infusions f sucroe /ar tificial csf into the late al entri l elicited drinking and antidiuresis lt ... to much less extent than imilar infusions of equiosmolal NaCl In thi study we made prolonged intr ventricular infusions f ucros /NaCl and mannit l/NaCl; control infusions wer equiosmolal pure mannitol and NaCl solutions Both the dipsogenic and antidiureti effects were transient during infusions f mannitol/NaCl sucrose/NaCl Plasma AVP initially rose rapidly and then fell in pons to thes infusions It dropped during mannitol/H_2O and rose markedly during hypertoni NaCl

The af composition ft mannitol/NaCl ucrose/NaCl revealed 10% lowering of the csf Na which could expl in why the dipsogeni and antidiureti ffects we much weak and les persi tent as compa ed t the ffect of hypertoni NaCl Changes in f Na ha been hown to be important i the regul ti f renal sodi um excretion (Ander on Dallman & Olsson 1969) and recently lso in the egulation f lt i tak (Wei ing et al 1979) Loweri g of f N doubled the con um tion f $NaHCO_3$ i sodium dep ived heep whe ea increa ed csf Na dimini hed $NaHCO_3$ uptak in the animal It i concluded that sodium en itiv recepto not only parti ipat in th regulation of w t balance but also in th control f salt balance

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M J McKi ley holds C J Marti F llowship f the N H & M R.C f Austr li

pH-dependence f recepto binding of insulin to rat adipocytes

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Binding f insulin to receptors i t adipocytes is approximately 6 times highe at pH 7 8 compared to the binding at pH ... A furthe increase in pH from 8 4 to ... causes light decr as in the binding of insulin. The im f this tudy was investig te the pos ibl reason f this change i binding

We have previously reported that about ... of the cepto -bound insuli i released from the cell as degradation products (Gliemann Sonne 1978) As the fractional degradation f recepto -bound insulin was the ame in the inve tigated pH-range (6 8 8 8) thi could not ... the observed diff rence It as also excluded that the diff rence an arti ... due to the use f iodinated insulin ... monoiodoins lin (Gliemann ... 1979)) ince iodoins li and native insulin showed the same pH-dependence ... ta binding xpe iments reveal ... the incr ased binding t lk line ... was due t change i ffinity the ... binding capacity Kine i xperime ts showed that the te f di soci tion w unchanged and that the degree f neg tive cooper ti ity was small at all investig ted pH alues Associ tion xperiments evealed that the increase in ffinity t alkaline pH could be accounted f by an increased soci tion rate constant

To te t the bi logical consequence f the change in affinity we measured as well glucose incorpo tion into the lipid pool as 3-O-methyl-glucose transport (Whitesell Gliemann 1979) The insuli dose-response curve f thes biological parameter were ccordingly shifted to the l ft t alkaline pH

In conclusion in th diabeti patient the acidosi will turn the cells insulin resi tant and thereby ende the sparce insulin eve more unserviceabl

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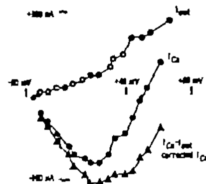
Early outward current and E_C in a ide H/ el ell er s

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Inward going Ca^{2+}-currents are important for acti ry in any excitabl cells Measurements of the equilibrium potential for these currents give va lues between +40 and +65 mV The Ca^{2+} equilibrium potential (E_{Ca}) i t least 120 V assuming that Ca^{2+}_i is 5x10^{-7} M. Several hypotheses exist to count for this d screpancy (Moolenaar & Spector 79) For exampl that an ea ly outward current interferes with the inward Ca^2 current

For soma of cell A of Hel aspersa with two independent screened microelectrodes voltage clamp studies were made using conventional techniques (Standen 1975) During depolarizing step the cell displayed a fast inward current and delayed outward current Ion substitution experiments has shown that about 2/3 f the fast inward current is carried by Ca^2 (I_{Ca}) I Na free Ringer the I-V relationship (● F g 1) and the time to peak for the Ca^2-current was obtained for 11 test potential I_{Ca} was then blocked by adding 15 mM Co^{2+} to Na-free Ringer (with 2 mM Ca^{2+}) and a I V plot was obtained again. All inward current was now assumed to be blocked and the current was measured after pulsetime equal to the previously found time to peak Ca^2-current for each test potential (O-Fig 1) Thi curve shows the ea ly outward current (I_{out}) that contaminated I_{Ca}

The equilibrium potential for early current is usually measured as the intercept of the I V



curve with the voltage axi (E tercept) I this case it is about +45 V for I_{Ca} E intercept (found by extrapol tion) shifts considerably to the right when I_{Ca} is corrected by subtraction of I_{out} I_{Ca} and I_{out} would be expected to unite at E_{Ca} However they became nea ly parallel t high potenti l I_{out} was insensitive to TEA (50 mM) or tetracaine (0 5 mM) 4-aminopyridine (5 mM) caused little or no inhib tion

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Tolerance to and dependence on morphine in the spinal cord

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It has been proposed that development f morphine tolerance and dependence may be due to functional chang in monoaminergic neurons in the brain (Way Ho Loh 1974) An lternative uggestion is that tolerance and dependence are related to changes in the sensitivity f opiate receptors (Cox 1978) Following transection of the spinal cord there is complete caudal degeneration f monoaminergi neurons (Carlsson Magnusson & Rosengren 1963) Transected rats were therefore used to investigate whether development f tolerance and dependence could be observed in structures deprived f monoaminergi i

Compl te pinal transections were made at the level f ve tebra Th_{10} in 10 mal rats Sixteen rats were used controls Afte 14 days f training in the test ituation 5 spinal ts and 8 controls were injected with morphine chloride 10 mg/kg i p At 12 hr interval for 8 days Tested for sensitivity to noxious stimul tion with the tail flick method all showed progre ive reduction in drug ffect (tole anc developme t) during the period f morphine tre tment

Naloxone HCl (1 mg/kg 100 min after the last morphine injection) induced igns f withdrawal both in pinal rats (pronounced twitching and j rking f tail and hindlimbs) and i controls (jumping in creased defecation) Withdrawal was not precipitated by morphine and naloxone i transected and in i tact rats not made tolerant to morphine

It was concluded that morphine tolerance and dependence can develop in the pinal cord without neuronal influence from th brain Functional changes in monoamin rgi neurons are theref re not necessary f these behavi al changes

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Do corticospinal fibres send collaterals to the lateral reticular nucleus ?

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The inferior olive and the lateral reticular nucleus (LRN) are major sources of cerebellar afferents Both nuclei relay information from the spinal cord and from supraspinal motor centres It has been proposed that these nuclei function as comparators; the hypothesis assumes that the ascending spinal information relates to the function of lower motor centres which are controlled by commands from higher motor centres and that the nuclei are informed of these descending commands by collateral action from the fibres to the lower motor centres (Clendenin et al. 1974) If such collaterals exist their stimulation in the nuclei should evoke the same action as impulses in the parent fibres We have tested this possibility with regard to corticospinal fibres and the LRN by comparing the extracellular focal synaptic potentials evoked in forelimb segments by electrical stimulation in the contralateral bulbar pyramid and in the ipsilateral LRN. Single pyramidal volleys gave a large negative focal potential in Rexed s layer VI but stimulation (200 µA) in the LRN evoked no trace of such effect in this region. The latter stimuli gave effective activation of afferents to LRN since a large negative focal potential was evoked more ventrally in the motor nuclei du to stimulation of ascending collaterals from C3-C4 propriospinal neurones which have bifurcating axons projecting both to the LRN and forelimb motoneurones (Illert & Lundberg 1978).

Our results thus fail to support the suggestion that corticospinal fibres send collaterals to LRN (Zangger & Wiesendanger 1973). These authors stimulated the spinal cord after degeneration of ascending fibres and postulate that the excitation evoked in many LRN cells was due to antidromic stimulation of corticospinal fibres. It can now be suggested that these antidromic effects might have been due to stimulation of the descending branch of the above mentioned C3-C4 propriospinal neurones which also project to the LRN.

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Effects of secretin and plasma Na ion concentration on pancreatic bicarbonate secretion

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Pancreatic HCO_3 secretion implies active equimolar transport of protons to interstitial fluid and is probably secondary phenomenon to such transport. Pancreatic HCO_3 secretion has been proposed to be due to pancreatic proton pump; the rate of proton transport would be determined by the electrochemical proton potential gradient sustained by the proton pump (Raeder et al. 1979). Hence extracellular proton concentration may also determine the rate of pancreatic HCO_3 secretion. An alternative hypothesis is that pancreatic proton flux is secondary to pancreatic Na^+ transport (Swanson and Solomon, 1975). Secretin would increase the electrochemical proton potential gradient generated by a pancreatic proton pump. Increases in plasma Na^+ concentration would augment Na^+ dependent proton flux.

To distinguish between these possibilities experiments were performed on 11 anaesthetized pigs. By raising intravenous secretin infusion rate from 1.4 to 2.70 C U /kg. b. w h^{-1} pancreatic HCO_3 secretion was raised from 79 _ 9 µmol/min to 309 _ 41 µmol/min (6 pigs). Pancreatic HCO_3 secretion varied in proportion to plasma pH during lowering of plasma pH by intravenous infusion of HCl and addition of CO_2 to inspired air. Calculated zero secretion occurred at plasma pH 6.96 _ 0.03 and pH 6.78 _ 0.02 during low and high rates of intravenous secretin infusion, respectively.

During constant intravenous secretin infusion (2.70C U /kg. b. w h^{-1}) plasma Na^+ concentration was increased from 144 _ 1 mmol/l to 193 _ 2 mmol/l by intravenous 20% NaCl infusion (5 pigs) and lowered from 139 _ 1 mmol/l to 113 _ 2 mmol/l by intravenous H_2O infusion (3 pigs). Variations in plasma Na concentration from 113 to 193 mmol/l did not increase rate of pancreatic HCO_3 secretion.

It is concluded that secretin increases the rate of pancreatic HCO_3 secretion. This effect may be mediated by an increase in the electrochemical proton potential gradient from pancreatic cell to interstitial fluid. Increases in plasma Na^+ concentration from 113 to 193 mmol/l does not augment pancreatic HCO_3 secretion. These findings favour the view that pancreatic HCO_3 secretion is consequence of active proton transport to interstitial fluid by pancreatic cell proton pump.

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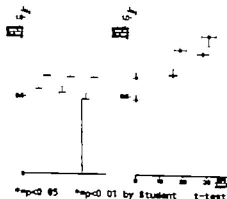
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Mechanical properties and metabolic rates of vascular smooth muscle from normo- and hypertensive rat

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A study has been made of the passive and active length-tension relations, rates of O_2 consumption (J_{O_2}) and of lactate production (J_{LA}) in vessels from aortas, 22 wk old (20-25) spontaneously hypertensive rats (SHR 313 5 g SE n=11) and age-matched normotensive Wistar-Kyoto rats (WKY 342 g n=10) Mean BP (tail artery ether anaesthesia) was 42 mm Hg higher in SHR Segments of the abdominal aorta were mounted on pins in glass chambers for registration of circumferential force J_{O_2} and J_{LA} (Hellstrand P 1977 Acta physiol scand 100 : 140) Adjacent portions of the same aortas were mounted separately for length-tension study At each vessel circumference passive and active tension (force/length) were higher in SHR Optimal circumference (OC) for active tension development was lower in SHR At OC active wall stress (force/area) was somewhat higher in SHR

The metabolic studies were performed at OC (P_{O_2} 150 mm Hg) Metabolic rates are given relative to dry tissue weight (30 5% of wet weight no difference between SHR and WKY) Fig (left panel) shows J_{O_2} (basal) in normal medium with 2 5 mM Ca^{2+} (A) and in Ca^{2+}-free normal (B) or K^+-high (100 mM) (C) medium. The result shown by rose-hatched bars. Right panel shows J_{O_2} vs active stress (P) in K^+ medium with 0, 0 5 and 2 5 mM Ca^{2+} (filled symbols) At all levels J_{O_2} is higher in SHR. The slopes of the relations, indicating the metabolic cost of contraction, is similar in both groups In



relaxed muscles in normal medium J_{LA} was the same in both groups and not dependent on presence of Ca^{2+}. J_{LA} was lower in K^+-high solution and decreased with increasing $[Ca^{2+}]$. No difference between SHR and WKY was seen In normal medium J_{LA} was 0 85±0 10 and in K^+-high solution (2 5 mM Ca^{2+}) 0 50±0 05 µmol/min g n=10 (WKY)

In a study of portal veins no difference in J_{O_2} or J_{LA} was noted between SHR and WKY

The metabolic differences found between SHR and WKY were most prominent in the basal J_{O_2}, possibly relating to increased active membrane transport. However the result may also be compatible with differences in muscle mass as fraction of tissue weight

C7

Skimmi g of mic aph s us s f l timat s of blood flow di trib tio i the d g kidn y

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D ri g enal va dil tion 15 µm mi o ph (Ms) i di at th t va l r o d ta e in r se p po tion taly m re i i th n in out r co tic l on Thi ld b du t f ilure f M to nt th i n r ff r t rt riol s in p po ti n to blood flow gg st d by p i d mp ri on f l t i t blood fl w d M co c nt tio (Cl n t l 1979) R ent mod l p rim nt (Øf) d t l 1979) indic t th t 15 µm Ms m y b kimm d l ng th i t l p la t ry (i l) Such kimmi g wo ld b pect d t b d p nd t o i l die t d t b l s pro oun d f m lle ph W th r for i j t d a mi tu f 10 µm d 15 µm M f timatio of bl od fl w i c ti l d m d llary n i D c t l kid y d i 4 kidn y with about 50% i d s l d t c p d d by i i f si f tyl b line. F ir d d ta i di ted th t 15 µm M d e tim t d in ti l fl w by bo t 20% a c mp r d t 10 µm M d i g co t l d by l th 10% d ri g v dil ti P vi e ult i di t th t 15 µm M und r timat i n rtic l flo by bo 60% du ing c tr l e su 15% d i g s - dilatio a comp d to n lte d onal fra tio l fl w ti at d f om l l I ntipy in upt k r te (Cl s t l 197 Thus both M sl s see t d r ti t de p co ti al fl w nd timat out ortical flo i c trol kid y 15 µm M to gr at r t at the 10 p Ms Althou thi under stim t i d c d d ri g s dilation th h g i th 10 µm and 15 p Ms dist ibutio mo g n b i ly gi fal d div rging ti t f h ng i on l blood flow

Sinc g nal xtr ti f th M w s 99 5% d only a i r fra ti of Ms w s lo t d in po tgl ul pilla i sl we n l d th t f lty int l di t i buti f bl d fl w s ti t d by M i due t kimmi g of M alo g th i l a A xp t d th kimming pp r t be l st pro u d with th s all ph d th l rg i l di t

R f n s

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C8

Reduced GFR i d hyd ted animal i the tubulo-glomerula feedba k

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Earli experiment have hown the exist nc f tubulo-glomerul r feedback ontrol that educ glomerul spill ry p ssure and glomerul r fil t tion r t t inc eased di tal d livery f fl id The ensiti ity f thi mechanism can l be e- t during diff rent physi logical onditions Ca pill ry mi rope fusion xperime t hav rev led that the plasma c lloid concent tion pe fusi g the mac l dens s an mod l t feedba k en- sitivity via hange in i t rstiti l pre ure and volume (PERSSON A E G & MÜLLER SUUR R & SELÉN G 1979)

The que tion ise if i d hyd ted animal the feedback ensitivity i i ea ed and f the e- duction in kidney GFR observed i thi ond tion can be xplained by an a ti tion of the t bulo-glomerular f edback control Dehydr ted animal (24 hrs without food and wat) were tudied be- for and afte saline volume xpansion (5% f B W) GFR was found t be signifi antly reduced d i g dehydr tion compa ed with control condition (0 96 ±0 08 ml/min and 1 49±0 09 ml/mi re pectively) During volume expansion GFR ros to 1 71 ±0 08 ml//min

In mi ropuncture experiments tubula top flow p sure was rec rded t diff rent r t f tifici l perfusion f the distal nephron The feedba k ha- act isti s were det rmined from the maximal d op i stop flow p ssure i per cent f the top flow p sur (ΔSFPI) and the pe fusion r t t whi h 50% of the ma imal top flow pres ur drop was t ined the turning poi t Duri g d hyd tion ther was a ignificant reduction i t rning poi t from 20 7 ± 0 8 1/ in t 16 3 ±0 4 1/min and an in- eas i ΔSFPI from 25 5 ± 1 8 t 34 2 ± 4 0 as pa ed with ontrol onditions indi ti g an in- e ed f edback sensitivity whil during volume expan ion t rning poi t was incr ased t more than 35 1/mi and ΔSFPI educed t 8 0 ± 3 3 indic ti dec ea ed feedback sen iti ity

T d t rmi if thi increased feedback ns iti it d i g d hyd tion gave ise t feedback l ed eduction i GFR di t l and proximal llections f SNGFR we mad i the same nephron Di tal collec t on with an i t t feedback loop gave ignificant lowe lue than p oximal llection with the fee back loop broken (32 2 ±1 9 1/ in and 37 3±2 6 1 /mi respectiv ly) I ont i ri no diff renc ould be d tected Thi re lt indi t that the feedba k ystem i ti ted t reduc GFR i dehy d ted animal

I concl on the duction i t t l kidney GFR th oc ur d hyd tion i t l t partly rel ased b the tubul glomerul feedback me hanism that ha g ined an inc ed ensiti ity du i g this ondi tion Thi lt is i gr ement with the theory that the pr ure-volume ondition f the i t r t urr rounding the ma l den c lls mod l t he en iti ity f the tubuloglomerul r feedback s ha ism

Ref enc PERSSON A E G & MÜLLER SUUR R & SELÉN G 1979 Am J Physi l 236(2) F97 F102

the Pattern Generator for Fictive Swimming in the Lamprey Ichthyomyzon unicuspis

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The lamprey spinal cord can be isol ted as an i vitro preparation and stimulated with bath-applied agents such as D-glutamate (0 3-1 2 mM) to produce fictive swimming rhythm (M.L T Poon 1 preparation; A.H. Cohen & P Wallén, i preparation). rhythm is recorded s regul bursts from ventral roots is centrally generated and preserves several features of swimming ntact lampreys. We have made an intracell l analysis of motoneurone (MN) activity long with experiments of applying solut ons locally to different cord parts. MNs were dent fied by firing them with pulses of depolarizing current wh l recording time-locked spikes in ventral root. Thei osc ll tions of membrane potenti l n time with the swimming rhythm, ranged i peak to-peak amplitude (exclusive of spikes) from only few mV to large as 18-20 mV. In most cases, the depolarizing phase MN was synchronous with the mai burst i the ventral root carrying its axon. lthough one MN has been recorded to date wh ch depolarized and f red antiphasically. I one MN the membrane potenti l level during oscillations (0 6 mM glutamate) was compared with the resting level during washing with sal ne beforehand and afterwards glutamate caused a net depolarization so that even the oscillation trough was 1-4 mV positive to the rest ng DC level. Cl- ions were i jected with hyperpol ri ing currents from KCl lectrodes to determine f periodic inhibition contributed to caus ng the oscillation trough i MNs (ventral root records gave timing cues). The trough phase could be reversed into secondary depolarization by Cl- injection and this was enhanced by hyperpol rizing the MN. Results were learest during brisk bursting output. These findings suggest the existence of nhibitory premoto interneurones which are bursting and helping to form the MN wave.

As many as 70 segments can be included i an in itro preparation yet only ca 4 re needed to produce bursting output (A.H Cohen & P Wallén i preparation). This llows type of experiment of chem cally stimulating local regions so that the rhythms different parts f the cord are cont rolled separately. I one ariation of this scheme the cord was di ded by aseline barriers into 3 pools rostral and caudal pools each enclosing ca 20 segments and middle pool enclos ng ca 30 segment. When glutamate was pplied to all 3 pools the burst ng output at all levels was rigidly coord nated i a l l manner with all level howing the same cycle period. If glutamate was then removed from the middle pool by wash ng it with saline the rostral and caudal parts each continued to produce bursting output but were uncoupled they showed widely different cycl periods and cross correl t on of thei burst t ming showed only weak s gns of nteractions. Thi suggests that the inter neurones subserving the tersegmental coupling probably project mainly for less than 30 segments. Indeed the results prov de some support for the swimm ng generator network being an iterated series of egmental generators with fai ly local interactions between segments.

Supported by NIH Postdoctoral F llowship (DFR) and SMRC project 3026

C14

_ _ _ _tio a d projection of olfactory
_ _ _ _ _ f r h r (Salmo l inu l

C.T _ _ELER

_ l t f Zoophy i l gy Un versity _ rway

fl h lfactory rg i iti t ro id nd b t f bil a id there t (Døv g t l 1978) Th _ f th lf t ry b lb to ti _ ith the timul re diff r- _ lly di t ib t Am acid gi _ i th l t l part f th bil id t medi l part firdi g i t the i t f tion lly d f rent popul tion _ ent ron recept ro re f und i on l f l lamell f rmi g _ rt i th l t ry a ity If _ lerti I t y b lb ary ont l p rati f th two types f _ euro t b xp t d Thi d f the rel ti fl en f iff re t lf t ry lamell th and di t ib ti f th b lb re po b lb surf pot t l we re from two po iti imultan ly l t lf t ry bulb Th lf t _ ll we uc ively remo ed l g diff re t t t gi T remo l f lam ll d red ti i the ampl t d f th bulb potenti l Th re p t bil id d t ami id di i i hed i pa ll l Mi dif f rem were b rved I on i di id l th mo t dal f th d re l lam ll rried predomi tly bil id iti re pt re d few b t till d t t bl ami id iti re pt re Th lt i di t th t th two typ f recept re re pre t ll th lamell but th i rel ti freq cy may ry Th re pt th ref re h t b rt d t b f re maki g y pti tant i th b lb Th fi di g how th f ti l ig ifi f th t i ro i g f primary rve f bre i th ro t l p rt f th b lb (Sheldo 191 d th re) Th y re l relev t f th di cu i how th p if ity f ew lf t ry receptor ll i d t rmi ed

Ref re

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C 11

The ffect o h bituati n and training on two and o e leg extension at e gth

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The classic co cept is that it is possi ble t recruit all motone rons simultane ously when performing a maximum voluntary muscle contraction (MVC) (Merton 1954) H wever resent determinations of the ratio (Ra) betw en MVC of extending both legs sim ltaneously and MVC of the two legs working independently show that un trained subjects are unabl to develop their f ll muscle strength during ex tension of both l gs when they are working togethe (Secher 1975 Seche et al 1978) Th aim f the p esent study i to investigate whethe the low Ra value of untrained ubj ct is due to a lack of habituatio or a lack f training
MVC was dete mined with both legs working simultaneou ly and with ach leg working al ne in 18 ubj ct t three oc casions ep at d by 5 w eks The 18 ub jects formed three gr ups 5 i a efe ence gro p 6 trai ed is metri ally by perfo ming 150 MVC 36 time with i ht and left leg separ tely a d th 1 t 7 s b je ts t ain d with 150 r pe ted MVC 18 times with b th leg At the fir t d te mination two d ne leg MVC er 3000 OO (SE) and 1660 92 N with a Ra value of 82 ± 2 6% At the second determinatio two leg MVC was 3200 ± 40 N hile one leg MVC had decreased to 1600 84 N Thus Ra had increased to 97 3 0% Irr spectively of the type of training p plied two and one l g MVC increased by 55 ± 9 0 and 40 5 0% resulting in a R value of 101 ± 3 3% During the tr inin period the reference group changed t o and one leg MVC by 2 0 and 14 1%
The results suggest that the lo Ra value of untr ined subjects reflects a relative imparement of the motoneurons when the legs are used simultane usly but that the imparement vanishes with ha bituation

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C 12

Effect of incre i g glucos loads on the rate of muscle glycog n resynthe is fte prolonged e ercise

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Muscle glycogen declines progr sively during submaximal e e cise requiring 60 - 80 % of maximal oxygen uptake (Vo_2 max) until total depletion at exh ustion (He mansen et al 1967 Goll ick et al 1973)
In view of the importa t role that gly cogen plays during p olonged exercise re-synthesis of glycogen tor in m cle a d liver must be an important component of the recovery process
The aim of the pres t investig tion was to study the effect of i cr ing glu-cose loads on the rate of mu cle glycogo resynthesis after prolonged severe e r cise Muscle biopsies were obt ined from 12 male subjects before and at va ious intervals during the first 8 ho rs of re covery after prolonged exercise (i o 70 % of Vo_2 max to exhaustion)
Three series of experiments were per formed with 4 subjects in each group The subjects were given 0 7 1 4 o 2 0 grams of glucose (in water) per kg body weight every 2nd hour of the recovery period The mean rates (±SE) of muscle glycogen resynthesis were 2 3 ± 1 2 4 6 ± 0 7 and 5 6 ± 1 4 mmole glucosyl units kg $^{-1}$ wet weight hr $^{-1}$ re pe tively
It is concluded that the rat of muscl glycogen resynthesi after prolonged ex r cise increases with incre sing oral gluco loads up to a certai level A glucose load above this level gave no further in crease in the rate of muscle glycogen re synthesis

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C 17

Mp. mian by cyclic AMP of the steroidogeni pa ters in the preovulatory rat follicl

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Previous studie from sever l laboratorie have shown that LH exerts biphasi effect on steroidogenesis in the preovulatory f lli le an initial stimulation of progesterone (P) androgen (A) and estrogen (E) production f llowed by an inhibition of A and E production (Lieberman al 1975) cAMP is generally assumed to be the intracellular mediator of the LH induced stimulation of steroid production. The aim f this udy was investi gate whether cAMP also is riggering the lat inhibition of A and E synthe is

Immature rat were pretreated with 8 IU PMS two days prior the experiments in order to induce f l licular development. Isolated preovulatory f llicles were incubated in chemically defined medium for 1-8 h with addition of ovine LH (10 µg/ml) or dibutyryl cycli AMP (dbcAMP 10 mM) The level f P, A and E were analysed by specifi RIA (Hill nsjö et al. 1976)

When the f llicle were incubated i absence f hormone there was an lmost linear increase wi h time in all steroids wi h A and E being the major steroid formed. Addition of LH or dbcAMP both produced marked increase in the rate f P accumula ion throughout the incubation Both hormones produced acute stimulation f A and E; however af 4 h of incubation the levels f A and E did not increase further To exclude adverse ff ct f steroids l ready accumulated during the first hour f incubation anothe serie f experiments were perf rmed in which f llicles transferred to fresh media af er 4-6 h for second incubation Also hese tudie showed that addition of dbcAMP mimicks the effect the ffect f LH both the acut timulation (high levels f P A and E i the first medium) and the la inhibi ion f A and E (high levels of P low levels f A and E i second medium)
The results f hi tudy re compatibl with the hypothe i that cAMP rigger the entire biphasi response to LH i the preovulatory follicl

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C 18

Apparent inhibition of th ADH secretion induced by intracerebroventricular vanadate

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It remains controve ial que tion whethe re bral recepto involved in the control of wate ba lance primarily are osmosensitive (V rney 1947) sodium sen itive (Ande on 1971) Howev effects obtained in response to the intracerebro ventricul (IVT) admin tr tion of rtain tumo lators and inhibito of active transmemb ane N transport suggest tha tion tran porting zyme activ ty may be sential fo the xcitation of ce ntral re eptor regula ing wate intake and the release of antidiureti hormone (ADH) from the neurohypophys (L Ande son 1978) The fore the recent di overy tha vanadate is poten in hibitor of Na, K ATPase acti ity (Cantl y al. 1977) made i of inte to study the ff ts on water balanc of IVT vanadate inf on in the conscious goat.

VO_3^- (0.1 µg/kg^{-1} min^{-1}) wa inf d in the late al vent icl ia permanently implanted cannula for 40 (nonhydr ted animal) or 30 min (hydrated animal) at te of 0 02 ml/min. In the nonhydrated goa the anadate wa dissolved in isotonic (0 15 M) NaCl olution whereas hype tonic (0.25 M) NaCl was d olve t in the prehydrated goat. Co responding infu ion of th pure NaCl solution rved ontrol
In the nonhyd ated animal the IVT administ ration of vanadate/saline induced on picuous wate di resis within 10 min. The w te diu outl ted the inf on period by 10 to 20 min. Du ing the control infu ions of imply isotonic saline the re al free wate l rance remained neg tive In the prehyd ted animal th control inf ion of per 0 25 M N Cl sol tion induced an inhibition of the wate diure within 20 min. The addition of vanadate to the hyperton N Cl lution p event d, or con de ably delayed thi antidiureti re sponse The the al free wate cl aran mained at th initially high lev l throughout the 30 min infusion period and f les 10 min af terwards
The re ults support the ide that timulation of N K ATPa ctivity in som manne is in l ed in the excitation of juxtace broventricular cepto regulating the ADH l se from the neurohypophy

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C 15

The redistribution of ACh receptor hotspots during synapse elimination

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During synapse formation throughout the nervous system it i common for an excess of synapses to be f rmed initially and for many of these to be subsequently eliminated The present study used the formation of ectopic neuro-muscular junctions on the adult rat soleus muscl to investigate wheth r elimination is random with respect to distance along the postsynaptic cell or whether for example a ynapse i more likely to be eliminated if it is lose to another synapse

The fibular nerve was transplanted onto the soleus muscle at a site proximal to the orig-inal endplate band and allowed to grow through-out the muscle fibres f r three t four weeks Th ol us (tibial) nerv was then cut and re-sected which caused the f rmation of neuro-muscular junctions by the fibular nerve in three days (Lømo & Sl ter 1978) We used ^{125}I labelled α-bungarotoxin and autoradiography to map the location of ACh receptor hotspots induced at new synapses

Initially the fibular nerve induced several hotspots along each muscle fibre After 21 days about 80% of these had been eliminated At 6 days after cutting the fibular nerve to induce ynapse formation the receptor hotspots were distributed at random along the muscl fibres. The di tribution of di tances between adjacent hotspot followed negative exponential curve If the elimination process operated at random one would xpect the distribution of hotspots at 21 days also to be random However thi was not the case Therefore elimination of hotspots was not a compl tely random process Rather our d ta support the idea that the probability that a hotspot will be eliminated depend on the di tance t its nearest neighbour

LØMO T & SLATER C R 1978 J Physiol 275: 391-402

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C 16

A likely physiological role of spermatozoal zinc

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The highly S S crosslinked mammalian sperm nuclear chromatin gives the sperm nucleus its high resis tance to different chemical and physical condi tions (cf Calvin & Bedford 1971) It is generally accepted that the ovum provides free thiol-enzyme systems of high efficiency to cleave the S S bonds

However, in human sperm nuclear decondensation has recently been performed without exogenous S S cleaving agents (Kvist 1979) It was suggested that the human spermatozoon may have an int insic free thiol system with ability when triggered to cleave intermolecular S-S bonds and thereby al low a rapid nuclear decondensation upon sperm en trance into the ovum Evidence was provided fo the concept that the nuclear chromatin decondensa tion ability(NCD ability) is reversibly inhibited by zinc derived from the prostatic fluid upon eja culation The NCD-ability could be reacti ated by zinc removal induced by conditions known to induce fertilizing capacity of ejaculated spermatozoa

The aim of the present study was to evaluate if spermatozoal zinc also would protect the sperm NCD ability from oxidative destruction upon sto age The NCD-ability of untreated and zinc-depleted spermatozoa was examined before and after storage for 24 h in a buffered salt solution with and with-out addition of zinc ions The proportion of spe matozoa with NCD-ability was significantly reduced in all groups upon 24 h storage almost totally(by 88%) in the zinc-depleted and by 44% in the un treated group In the zinc-depleted and zinc sup lemented group the reduction was only 36% a val not differing from the untreated and zinc supple mented group (38%) Other differences significan (p 0 05)

The results are in agreement with earlier repor (cf Kvist 1979) that human spermatozoa lose their NCD-ability upon storage an effect that also can rapidly induced by o idative agents eg Cu^{2}

The results indi ates that spermatozoal zinc de rived from the prostatic fluid upon ejaculation mi ht be of physiological importance to protect th sperm NCD-ability in man Such a protection would also be manifested as a reversible inhibition of the sperm NCD-ability which in turn creates a need for its reactivation by depletion of sperm zinc in the female genital tract In favour of these sug-gestions are the ea ly observations of Chang and Austin in 1951(cf Chang & Hunter 1975) that the spermatozoon has to remain in the female genital tract for several hou s to gain its fertilizing ca pacity and that the spermatozoa release zinc du-i n this period(Gunn & Gould 1958)

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The vagal control of the feline pylorus

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The pyloric motor activity wa tudi d by
ing the change of an xternally pplied flow f
body-warm saline acros the phi cte t on
constant perfusion pr ssure in cat i chloralose
anesthesia (Edin et al 1979) The rvical
vagi were cut unilaterally and the distal and p ox
mal end spectively were inserted int ri g
electrode and stimulated le trically Whe H
stimulation was given in the ent direction using
high threshold paramete (8 10 V 5m 8 Hz
according to Martinson 1965) bipha ic motor
response with an initial dilatation followed by
long-lasting contraction wa obtained Whe
vagal stimulation wa give afte closure f the
pylorus by infusion of norepinephrine (0 01 0 02
μg/kg b /min i.v.) dilatation wa indu ed
indicating that the vagi serv y both xcitat ry
and inhibitory fibe to the pylorus At ff rent
vagal nerve stimulation there wa pylori on
traction, hich disappe red afte divi ion of the
contralateral vagu indic ting vag vagal reflex
Pretreatment of animal with tropine (0 2
mg/kg) and/or ismeline (2 mg/kg) did not block
the motor responses ndu ed eithe by fferent
efferent vagal nerve stimulation Howeve hexa
methonium (25 mg/kg i 40 mg/kg h
according to Goyal & Ratta 1975) ompletely
blocked the pyloric ontraction at both fferent nd
efferent nerve timulation. Howeve the dilata
tion t fferent stimulation pe i ted unaffected
The dilatation wa pre ated if hexamethonium wa
followed by tropine (0 2 mg/kg) in cat with in
tact planchni nerve The sult indi te that
the vagu exerts both itatory and inhibitory
control of the feline pylorus via non-choline gi
on-ad ne gi nerve fibe F rthe xperiment
have indicated that peptide (akephalin, va
tive inte tinal peptide) may be involved in thi
vagal ont ol (Edin t l 1979 b)

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Supported by the Swedi h Medi al R se ch
Counc l (17X 05220) The Göteborg Medi al
Society and M di l F culty Unive ity of
Götebo g

Ischemic l ad lect ophy i logical evi
ce fo l glutamat natur l trans
tt r f hippocamp l CA3 pyramidal ll

I.I. SKREDE & D MALTHE SØRENSSEN

Norwegian Defenc Res rch E tabli hme t
Division f Toxicology Kj lle N rway

Several lines f evidence indicat that
L-glutamate (GLU) may be citat ry
transmitter in hippocampus Howev r
as requi ement f transmitt identi
fication is only pa tly tisfied th
release from p synaptic lements in re
sponse to physiol gical timul tion We
therefore tudi d the l f D-
[3H]-aspartat (ASP) in r spons t l c
trical timul tion f two well defined
neural branche from CA3 pyramids the
Schaffer coll ter l nd th hippocampo-
septal projection through fimbria D-ASP
was used ma ker f L GLU
Experiment pe formed on the
transverse hippocampal slic (Sk de &
Westgaard 1971) and on new slice pre-
paration consi ting f dorsol teral ep-
tum with the ing fimbria As for hippo
campal slices fib volleys ingle and
multiple unit discharg s and evok d po
tentials wer recorded from th septo
fimbrial preparation
Whil ld l ctive lectri l stimu-
lation f CA1 citatory fferent fib
(Schaffer ll ter l) well s f th
CA3 fferents to dors l t ral septum
through fimb ia r l ased D-ASP in ti
mulus dependent manne Th slices we
p lo ded with adiolabell d amino acids
and pla ed in a superfusion cell before
timul tion st rted
The stimulus evoked r leas f lfilled
s veral of the requirements f r neuro
t ansmitter ide tification in as much s
it wa Ca^{2+} depe de t and pe ifi A
presynaptic fibr volley wa present I
both high (1 5 mM) and low (0 1 mM) Ca^{2+}
but D-[3H] ASP was rel s ed t high C
only W ith GABA no L leuci w r r
l ased in re pon e t stimul tion (25 50
Hz)
Howeve D-ASP does not discriminate be
twee gl tamergic and sp rte gic t r
inals but e at inv stig tions from our
l bor tory allow f r uch discrimination
For xampl , both surgic l and chemi l
l sion of the CA3 xon collat ral wer
ccompanied by a significant decrease in
both high-affinity upt k of L GLU and i
th endogeneous conce tration of L GLU in
lateral septum with no changes in endogen
ous level of L ASP (review Fonnum t al
1979)
Thus in conclusi n our r sults, which
ar consi tent with Dale principl fa
vour L-GLU and not L ASP s neur t ansmit
t f the hippocampal CA3 eurones

R f r nc

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C 19

ATP per se inhibits non cholinergic non adrenergic transmission in the rat urinary bladder

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The excitatory transmission in the urinary bladder appears to be predominantly non-cholinergic non adrenergic(Ambache & Zar 1970) Since ATP contracts the detrusor muscle and since ATP may be released during nerve stimulation the atropine resistant transmission in this tissue has been considered purinergic (Burnstock et al 1978) Recently however it was shown that purines including ATP inhibited the contraction response to transmural nerve stimulation(TNS) in the guinea pig and rat urinary bladder(Dahlén 1979) The assumption that the inhibitory action of ATP is caused by metabolites eg adenosine(Brown et al 1979) was tested here

Rat detrusor strips were placed in organ baths containing Krebs Henseleit s solution gassed with 5 % CO_2 in O_2 and kept at 37°C Responses to TNS (5 Hz 0 5 ms supramaximal voltage 10 pulses at 1 min interval)or acetylcholine(ACh) were recorded isotonically(load 0 5g)or isometrically(tension 5mN)

ATP (1 1000 µM) and adenosine (1 1000 µM) dose-dependently and reversibly inhibited both the contraction response to TNS and the contractions caused by ACh(50 µM) or direct smooth muscle activation by long pulses(5 Hz 15 0ms) Treatment of the tissue with adenosine deaminase effectively annulled the inhibition by adenosine while the inhibition by ATP remained unaltered Furthermore the stable ATP-analogue β-γ-M-ATP(1 1000 µM) also inhibited the contraction response to TNS and ACh being at least as potent as ATP (Fig 1)

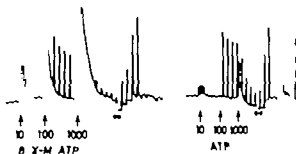


Fig 1 Effects of cumulative doses of β-γ-M-ATP and ATP(concentrations in µM)on contraction responses to TNS in the rat urinary bladder in vitro Drugs added at arrows bath fluid changed at dots

These findings seems to be at variance with the concept of purinergic excitatory transmission but suggests the presence of postjunctional purine receptors that inhibit non cholinergic non-adrenergic transmission in the rat urinary bladder

Suppoted by grants from the Swedish Medical Research Council(4342) and Karolinska Institutet

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C 20

Possibl role f prostanoids in the regul tion f smooth muscle activity in the human ov duct

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By the use of an in vitro model wh ch permits mechanical separation f muscl l yers with di par to orient tion we were abl t reveal differenti tion f adrenocept r responses f smooth muscl in the isthmi portion of the human oviduct a region which is believed to play k y rol f r the proper timing of ovum transport (Lindblom et al 1979) The present repo t de l with the possible significance of prostanoids i the control f contractile acti ity in the human oviduct

Tissues from fertile women were obtained t operation The isthmic portions f the oviduct were rapidly excised and immersed in chilled oxy genated Krebs-Ringer bicarbonate buff r Muscle strips ith cross sectional areas f appro 1 sq mm and length of about 4 mm were d ssected from the rious muscle layers t the ampull ry isthmic junction (AIJ) and the utero junction (UTJ) The contractile properti s of these spec mens were then studied in organ bath expe iments

Addition f prostaglandin E (PGE) to the rgan bath caused a concentration-dependent inhibition of the spontaneous phasic acti ity in the circular muscle layer f both the AIJ and the UTJ but i duced an excitatory response in the external longitudinal layer In the innermost longitudinal muscl layer the effect of PGE was dependent on the phase f the menstrual ycle an ex ita tory response was constantly achieved i the periovul tory period whereas in the ther phases of the ycl there was an inhibitory ffect PGF on the ther hand evoked powerf l e cit tory response in ll muscl layers independent of the cycl phase Prostacyclin (PGI) contracted the out r longitudinal muscul ture in the same manner as did PGE and stimul ted the innermost l yer in all phases of the menstrual cycl PGI however had very weak ffects on the i ter medi t c rcular layer

Indomethaci an inhibitor f PG ynthesi aused concentr tion-dependent inhibition f contractil acti ty in ll types f st ips which could be reversed by the addition f small amounts f PGF Furthermore it was found that ex it to y responses produced by the -adreno-ceptor agon t phenylephrine were unaffected by indomethacin

In conclusion the results demonstr t the diff renti ted di tribution of PG recept rs i the smooth muscul ture f the human oviduct Moreove the dat suggest that endogenous yn thesi f prostanoids i prerequisit for spontaneous acti ty in thi type f smooth muscl under in tro condition

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Afferent arteriolar diameter in two-kidney one clip hypertensive rats

L MYKING, J M. IVERSEN and J OFSTAD

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The entrance of microspheres (MS) from afferent arterioles (AA) into glomeruli (GL) has been used to estimate mean diameter of the AA (Myking et al 1978). The method is here used in the study of 8 normal rat kidneys (NC), 6 non-clipped (NC) and 6 clipped (hC) kidneys from 6 clip-hypertensive rats 70 days after clip application and in 6 similar pairs of kidneys (PC and pC) 70 days after clip application and 12 days after clip removal

The following results were obtained

	SAP(mmHg)	RBF(ml/min/g)	d(μm)
E	154 ± 4	2 73 ± 0 23	19 2 ± 0 4
E	212 ± 15	2 80 ± 0 39	16 5 ± 0 2
E		2 55 ± 0 53	20 8 ± 0 6
E	133 ± 13	3 19 ± 0 28	17 1 ± 0 6
E		3 09 ± 0 40	19 1 ± 0 4

Given as mean ± SEM and the entrance curves of MS from AA into GL from which the d-values have been calculated are shown in the figure

Application of the clip induced significant systolic arterial pressure (SAP) increase which in turn resulted in significantly increased d ($p < 0.05$) in the clipped kidneys (hC) and significantly reduced d ($p < 0.0002$) on the non-clipped kid (NC) to maintain unchanged renal blood flow (RBF). Removal of the clip allowed SAP to return to normal values also with complete regression of d back to normal on the clipped kid (pC). In the non-clipped kidneys (PC) d was not significantly larger than for the corresponding kidneys (HS) during clip-hypertension (p 0 2) and d was still significantly lower than for the normal control ($p < 0.02$)

This indicates that perfect AA RBF autoregulation is present after 70 days on the clipped kid whereas hypertension induced diameter change of the AA in the unclipped non-clipped kidneys was not abolished by normalisation of SAP for 12 days. Since these kidneys still have not reduced RBF compensatory vasodilation must have occurred somewhere else in the kidney

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rel entrance of microspheres into glomeruli

0 — 0.8 — 0.6 — 0.4 — 0.2

10 12 14 16 18 20 22 24 26 28 30 μm microsphere diameter

normal controls

CLIP-hypertensive

post-CLIP

C 26

Heterogeneous blood flow ... l ... the tourniquet shock

TIITSLOTH IRKENS

Institute Physiology University of Bergen Norway

The effect of bil l h ndlimb luni the di ibuti f nal co ti al land flow l ti d i deg Loc l Lanl flow i cert (OCP) i inner morta (ICP) as by 6 Lathium l ot d d t g hydrog g ... l blood flow f ll ig ifr ... while hema oc i (CT) on to 47 du ing th 4 h iq p iod ... the l f th quete ... ar i l blood p (AP) f ll ... t 73 f ea l h OCP and ... I operation ly du d tev d ... f oa l i ased 88% ... thy in rai an blood low i ... ser g l ly has d ... hindlimb l i and f ... f th tourniq most f que ... when it had ed k dly ... only found ari g d hyd en ... 197) d endo ... ide hif i gl l e- ... ta no comp d ... sponding i i total l ... pte bl king h pa ... t epp the ppe ... i thi pe i d owava ... f les l flow lee

ah y f ppli f th eral q b f h d ed i thi pha po bly d d by an d rv to l OCP and ICP f ll th an eg i g t ar iqu t h k P cby i ra tan blood fl h d i l co mo f q ly high h l bef m t did l kedly

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C 23

The effect of barbiturate anaesthetics on motoneurone depolarizing responses to glutamate and DL homocysteate

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Intracellular recordings from cat spinal motoneurones have revealed differences in the mechanism underlying depolarizing responses to excitatory amino acid analogues (Engberg et al 1979) In this study we have investigated the interaction of barbiturate anaesthetics with the depolarizing responses of motoneurones to glutamate and DL homocysteate (DLH)

Intracellular recordings were made on motoneurones in anaemically decorticated cats The amino acids were applied iontophoretically from extracellular barrels surrounding the recording electrode The barbiturates pentobarbitone and thiopentone were either injected slowly I V (4 15 mg kg $^{-1}$) or applied iontophoretically Blood pressure was carefully monitored throughout and experiments were rejected where vascular movements had clearly caused instability of the recording Membrane input conductance (G_M) was measured by injection of constant current pulses through the intracellular recording electrode

On resting neurones I V doses of barbiturates decreased spontaneous synaptic activity and caused a 2 4 mV hyperpolarization with no change in G_M

Long lasting glutamate-evoked depolarizations (accompanied by a slowly increasing G_M) were usually decreased in size by the barbiturates though occasionally increases in response size were seen In both cases however the barbiturat reversed the large increase in G_M evoked by glutamate

DLH evoked depolarizations (accompanied by a stable decrease in G_M) were always attenuated by the barbiturates The depression of response size was greater than that seen for glutamate when the two agonists were tested consecutively The barbiturates however caused no change in the conductance mechanism underlying the response to DLH

We have previously suggested that the high G_M state evoked by glutamate may reflect low affinity glutamate uptake (Engberg et al 1979) Were barbiturates to depress this uptake this would explain the barbiturate induced reversal of the high G_M state

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C 24

Spontaneous changes in membrane properties of Purkinje cells

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Institute of Neurophysiology University of Copenhagen Denmark

In recent experiments it was found that the cell membrane of Purkinje cells has pacemaker properties (Hounsgaard 1979) The aim of the study presented here was to investigate the intrinsic adaptive and slow oscillatory changes in the membrane properties of Purkinje cells

Intracellular recordings were obtained from Purkinje cells in cerebellar sections prepared and maintained in vitro as described previously (Yamamoto 1973)

Approximately half of the Purkinje cells were active only intermittently Silent periods occurred at regular intervals and were initiated by a sudden spontaneous hyperpolarization of more than 5 mV During the silent period the membrane potential and the membrane conductance decreased in parallel TTX (10^{-6} M) reduced the amplitude and increased the duration of recorded action potentials but did not block the slow oscillations in membrane potential Substituting Ca^{++} with Mg increased the amplitude of the slow change in membrane potential and obscured the normal spike generating mechanism.

The cell membrane adapted slowly to an injected current The firing rate increased gradually during a depolarizing pulse and reached a maximum after more than 1 s A preceding hyperpolarizing pulse slowed the increase in firing rate during the depolarizing pulse This effect was correlated with the duration of the hyperpolarizing pulse in the time range of 0 1 s to 2 s

The results show that the membrane propertie of a Purkinje cell at a given time is influenced by the membrane potential during the preceding seconds This indicates that synaptic inputs are integrated over times as long as several seconds in Purkinje cells. In addition the effect of synaptic inputs may be modulated by intrinsic oscillations in membrane properties

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C 29

Tad flow characteri tics and reactivity to nor
ervaline as tudied i vivo in induced mammary
neoplasia

L KESS, E. TVEIT & R. HULTBORN

Departments of Physiology Surgery Hi tology and
Radiotherapy University of Göteborg Sweden

A large number of tudies on tumour vascula
ture has been performed upon transpl nted tu-
mours with morphological methods nd it ha often
been observed that tumours have a very irregula
vascular pattern with large vessels not diff ren-
tiated into arterioles and venules and with poor
development of smooth muscle

In the present tudy the regul tion of blood
flow of induced and autotransplanted DMBA (dime-
thylbenzanthracene) at mammary neopla ia s
compared to various histogenetically li ked and
normal tissues has been investigated

Cardiac output and blood flow distribution were
studied by traditional microsphere tracer tech-
nique with sphere diameters of 15 and 25 µm The
trapping of the spheres i the tissues has also
made it possible to visualize by gamma-autoradio-
graphy the flow distribution withi the tumours
Histological examination nd fluorescence micro-
scopy for nerve endings have been performed

When nutritional blood flow under "resting con-
ditions" in standardized nembutal anaesthesi was
induced as well as autotransplanted DMBA tu
3-7 times higher than that of ski and ke
tal muscl The larger the tumour was the lowe
flow per unit weight During noradrenaline i
fusion blood flow wa markedly reduced i both in-
duced and autotransplanted tumours whil blood
flow to muscle was not at all affected and to
kin only slightly ffected by the given doses
Correl tion of re ting flow to the degree of dif
ferentiation revealed that tumours with low grade
of differentiation had lower blood flow than
thos with high grade of differentiation

Monoamine fluorescence icroscopy for nerve end
ings have not shown any drenergic nerve termi
nals i vessels situated i the tumo tissues A
moderate change i body temperature produced a
significant increase in skin blood flow but if
anything a decrease i tumour blood flow Four
times more blood passes through rteriovenous
shunts up to 25 µm i diamete to the lungs i
tumour bearing rats than i nontumour control
and t least 30 pe cent of the blood flow to the
tumours pas through shunting ve sels larger than
15 µm The gamma-autoradiography technique reveals
modul high flow areas surrounded by low flow
rea Most of the h gh flow moduli are situated
towards the periphery

From the present res lts upon DMBA tumours it
seems as the blood vessels supplying the tumour
cap llary beds have relatively low vascular
tone a compared to the host organ are regulated
by the metaboli needs of the tumour cells and
are poss bl to affect by humoral constricting
timuli The most abnormal features of the tumour
blood flow regulation re the lack of nervous in-
fluence upon the blood vessels and the high
amount of blood which is shunted past the nutri
tional part of the cul bed

C 30

Effect of impaired r flow following
...al ischemi

L SIMWONICZ

Institute f Medical Phy i logy Dept A
University f Copenhagen and Dept f
Anesthesia Hvidovr Unive ity Hospital
Copenhagen Denmark

Studies of eperfusi f the b in f
...ar ischemi have suggested that osmoti
swelling f endotheli l cell (Ames t al
1968) erythrocyt aggreg ti (Fisch
Ames 1972) or depolarisation f rterio-
lar smooth muscl (Wade et l 1975) may be
responsibl for impai ed po tischemi e-
perfusion

Carotid infusion f line ith labeled
...ol was used t estimate local perfu-
sion coeffi i ts f the ipsil t l hemi
sphere by scintill tion l ting b in
...ent of trace t its concentration i
...sagittal sinus C ebrovascul esi t nce
...) the ti f perfusion tes t
...sion pres res i th ange between 60
and 120 mmHg was lcul ted (Fig) At 100
mmHg infusion p es ur CVR i control
... ... aged 1 6 mmHg/ml 100g min
... ... f total ce eb l ischemi
... i incr as f CVR found Narrow l
... t blood from th b i t the
start f ischemi did not ignifica tly
affect the postischemi CVR P i chemi
treatment with anhit l i hyperosmol
...tities incr ased plasma osmol lity by
4 mosmol/l but f iled t ffect CVR f

i chemi Th es lts indicate th t 10 mi
f ce eb l ischemi incr ases ce ebrovas
cul esi tance ve l f ld Compone ts
f t gnant blood nd osmoti swelling of
the endotheli l cells we found not t
contribut to the increase Art riol v
soconst iction ppeared to play maj
rol i th po ti chemi incr se f CVR

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C 27

Int r titi l lloid osm ti p ur in p ti t with hyp p tei mi

H NODDELAND S MIDTBO RIISNES
A H O FADNES

In tit t of Phy i l gy U iv rsity f B g No w y d Medic l Depa tm nt A H uk land Syk hus B g n Norw y

It i well kn wn linic l ob r ti n th t p tient with hypop t in mi d t d velop d m u til lbumin t ti in pl sma i b l w 20 g/l F d s (1975) r p t d that a d ction of i t titi l p ot in on nt ti n is n import nt f tor in pr v nti g de f rm ti n du i g d v lopm t of ami cl id n ph is in at Our q tio wa wh th r th am m hani m w po ible f r th ed m p v tio i p ti t with hypopr tei emia

Th m t i l includ s t l gr p of 10 h lthy volont a d 13 p ti nt with ph otic y dr m m with d m d m with ub rm l pla m p t in c t atio nd n d m I t titi l olloid o m ti pr i b ti (π_i) w s m a r d by th wi k te h iq d s ibed by F d & A kl d (1977) 4 6 wi k w r impla t d i ub uti o th r x d th kl Aft l h th wi k w m v d a d th fl id withi it ll t d by t if g tio i liquid p r ffin A ll id s t m d f 5 µl s mpl w d t m π_i Coll id sm ti p ss ur w als d t rmin d i pl (π_p) btained by v nipun t

In th gr p of n rm l v l t e s f u d m π_p f 29 0 Hg mean π_i th x f 14 5 m Hg a d e π_i th kl f 11 0 mm Hg At l p d to hypop t i mia th r d cti in π_i u til th le l h r d oc ur at π_p a und 15 mm Hg At thi l el i v ag d 5 m Hg o th ad 3 mm Hg th a kl A f rth r d tio f p did t low r π_i sig ifi atly

The p s t r ult i di t that th w h t of i t titial p t i c se d cti f π_i th t o pe tm f bo t 70% f th d ti m in π_p ad th by s m t b th majo d a pr ting fa t i p ti ts with hypoprot i i

R fe

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C 28

Developme t l aspe ts f pulmonary lipolyti tivity i the g owing bbit

E HIETANEN J HARTIALA & M SIMBERG

Depa tme ts f Phy i logy Unive sities f T rk and Kuopi Fi land

The pulmonary urf tant y the i i one f the rucl l fact rs in the init tion f the respi a- tion The formation f dipalmitoyll ithi f om f tty acid may form t limiting t p i thi ynthe i Pulmonary lipopr t i lipase enzyme rel ases f tty acid from chylomi rons and VLDL lipoprot ins (Heinemann & Fi hman 1969) Due t the presence f tive lipoprot i lipas i ap- ill ry nd thelium and becaus f the l rge vascu- l bed (Scow t l 1976) lung might be x- pe ted t have high lip lyti apa ty (Hamosh & Hamosh 1975)

In our ea li r tudy we found th t pulmonary lipoprot in lipas a ti ity had peak ios imme- diat ly preceding the bi th f llowed by t amsi t decrease (Hi tanen & Harti la 1979) The hang were companied by respe tive change i the pulmonary phospholipid content Our p en lm was t study the development f pulmonary l po- lyti activity using pe fused lung as model Both foetuse and neonat l abbits were sed Krebs-Ring s bi rbonat buff wa used perfusion medium whi h wa upplems ted with Int a- lipid as ubst t Sampl we take during 2 h perf ion and free fatty acids were meas red according t F lholt t l (1973) The lipolyti activity was f l ly linear during the 2 h per- fusion The tivity was highest i th newborns (38 6 5 5 µmol FFA/h); decre ing t 24 4 2 4 i 1 day old neonat and increasing lowly du ing the first we k postnat lly

The lts uppo t the i itro d t that the ma ked chang s t th time f birth i the p lmonary lipolyti acti ity po ibly r l ted t the factant synthesi Thus eve also change i the lipolyti a ti ity ight yi ld di turbance i the urfactant ynth i and delay the i itia- tion f the re pir t ry function

G ant NIH (No R01 ES-01684) J V ini ad Y J hasson Found tions

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Changes in arterial blood flow measured
[illegible] in humans by new bidirectional
pulsed ultrasound doppler blood velocitymeter

[illegible], [illegible], J -E [illegible]
[illegible]

Institute of Physiology and Informatics
University of Oslo, Norway

A new heterodyne ultrasound doppler blood velocitymeter has been constructed (Hatteland [illegible] 1979) The instrument operates switchable in two frequencies (1 5 Hz and 6 MHz) and [illegible] is pulsed or continuous mode [illegible] filters have been incorporated in the instrument in order to eliminate doppler signals from wall movements A heterodyne output is generated by mixing the two quadrature components around an adjustable baseline frequency

In our experimental setup the doppler instrument is interfaced to cassette tape recorder and to microprocessor system The computer is programmed to calculate spectra every [illegible], instantaneous mean velocitie from the spectra, time average for each cardiac cycl of these mean velocities maximum and minimum velocities, etc (Will 1977) As long as the cross-sectional area of the vessel is constant the mean velocity i proportional to flow

[illegible] instrument has been used to measure changes in arterial blood flow to the brain ([illegible] and Walloe 1979) to skin areas to the [illegible], and to the uterus

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D2

[illegible] of sprouting f presynapti nerve [illegible] by [illegible] stimula ion of the post [illegible] neurones [illegible] partia denervation

[illegible] A [illegible]

Institute of Physiology University f Osl[illegible]
Norway

We have recently shown tha in the guinea-pig superior cervical ganglion the rate f sprouting [illegible] residual preganglioni axons [illegible] partial denervation is increased by period pre-ganglionic stimulation at the time of partial denervation (Maehlen [illegible] 1979) The mechanism [illegible] is effect i unknown In principle the effect of preganglionic stimula ion [illegible] be [illegible] by activity ther in the preganglioni [illegible], the gangl on cel or both The purpose of the present work was to examine whether postganglioni [illegible] has any effects on the ra sprouting

Under pentobarb tal anaesthesia the inferior postganglionic nerve which largely free of preganglionic axons was stimul ted with 5 pulses [illegible] at 20 Hz every 25 [illegible] immediately after partial denervat on. The peripheral [illegible] of act observed during stimulation [illegible] lied to the egion suppl ed by thi nerve [illegible] days the gang ion was removed for intra-cellular ecord ng Neurones with axons i the inferior postgang ionic nerve wer identif ed by [illegible] stimul ion, and the number f residual preganglioni axons contributing [illegible] to each neurone was judged by graded [illegible] f the preganglioni nerve There was an increase in the number f steps in the synapti responses in neurones whose axons had been stimul ed during the initial operation both compared to them stimulated neurones in partially denervated control ganglia (the inter polated median increased from 3 1 to 4 0 the ypical value is normal ganglia is 2 3) and compared to unstimulated neurones whose axons emerged in the superior postganglionic nerve in the same ganglion (0<0 001 Wilcoxon van Eltern test)

As in normal ganglia less than 3% f the neurones showed synapti potential upon anti dromi timulation f thei axons in the inferior postgangl oni nerve Therefore prolifer tion f collateral connexions between ganglion cells did probabl not occur

We interpret the increase in the number of synapti inputs to ganglion cell as evidence for sprouting f residual preganglioni nerve terminals Thus period hyperactivity in sympathetic ganglion cell appears to have retrograde trophi fluence on esidual pre-ganglioni axons after partial denervation.

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C 31

Substance P release and effects in the eye

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The undecapeptide substance P (SP) discovered by v Euler and Gaddum and widely distributed in the nervous system has been proposed as a sensory transmittor It has been suggested that SP release from peripheral nerve endings causes the vasodilatation and permeability changes that characterize axon reflexes (see Lembeck 1953)

In the eye at least two mechanisms seem to be involved in the response to various irritants one involves the prostaglandin system the other a neural component

In a recent study (Bill et al 1979) we found that both electrical and mechanical stimulation of the trigeminal nerve in albino rabbits distal to the Gasserian ganglion caused release of a SP like immunoreactive agent (SPLI) Both nerve stimulation and local injection of SP (0 1 or 1 0 µg) caused miosis a rise of the intraocular pressure (IOP) and a breakdown of the blood aqueous barrier Indomethacin modified but did not prevent these responses The effects of nerve stimulation were rather variable

We have now found that stimulation of the nerve close to the ganglion and of the ganglion itself gives more reproducible results in rabbits and that SP has effects that seem to vary between species

In the cynomolgus monkey cat and guinea pig 1 0-40 µg SP injected into the anterior chamber had little or no effect on the pupil size while 1 0 µg SP produced an intense and prompt pupil constriction in albino rats

Repeated mechanical stimulation of the nerve and the ganglion for 10 or 20 min resulted in high SPLI concentrations (174 666 pg/ml) of the ipsilateral aqueous humor (15 68 pg/ml) of the contralateral and (30-106 pg/ml) of the plasma The volumes obtained from the eyes varied between 40 and 150 µl Equal SPLI concentrations were obtained 10 and 20 min stimulation

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C 32

Stiffne h ges d ring th late t period of gl muscl f bre sol ted from the frog

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The f rst ten ion response follow ng th t mulat on of vertebrate sk l tal m scl f bre the latency rela t on (LR) which i th mall reduction of the rest g tens on prior t the developm nt of th ontra t l forc Durn g activity muscle become on iderably t ff tha t re t (Buchthal 1942) Th incre s could be sociated w th the ttachment of cro s-bridge t th th f lam t H s lgrov & H xley (1973) ha found evidenc fo movement of cross-bridges tow rd the th f lament durin g contraction. The incre s th t ffne beg t bout the same moment the LR (H ll 1950) I th present in estigation the purpose w t loc l th t me of th incre se more precicely The tiffne w me sured by subjecting the fibre to a small (0 3 nm half sarcomere) rapid length change bef re and t ariou t m s f r th stimulation A the time resolution w l m ted by the rise time of th step (0 4-0 6 m), the xperiment were performed i the temperat re range 3-15°C t wh h th performed i the temperat re range was suffic ntly low F r f bre tim coure of the LR was suffic ntly low F r f bre held at sarcom re length 5 3 µm and t tempe t of 10°C the f r t change in tiffnes occurred 5 5 m after stimulation nd 1 5- m ft r the onset of th LR The stiffness increased ea ly line rly with t me nd when the tension trace w s crossing the re ti g ten on lev l the stiffness had increased 45% over th t ng v lue At all temperat re stud ed the tiffne incre ed after the onset of the LR F r fibre held t 5 3 µm and at a temperature of 10°C or lower there wa delay between the time of increase i tiffne s nd th time i wh ch the rat of t ns on drop t rt t decrea e corre pond ng to 1 5-3 m At tempe t re of 14 15 C n such d l y could be detected nd the t me f incre se st ffne nd th fle ion point showed th sa depen denc on sarcomere length Based on th temper ture dependence of the t ourse of th LR Ha gen (1979) rgued that th reduct on of th t of te on drop depend nt on C^{2} d ff ng nt the one of overlap b tween th thick nd the thin filament Th coincidence t ll sarcom re l gth between th ti of thi event nd the ncre up tiffne ndic t s that both latt depende t on C^{2} d ff on nd might be due t tta h ment of ros bridge The delay betw en the onset of th LR nd th nc se in t ffnes nd t that the LR i not depend t on C^{2+} th lap zon nd is compat t bl w th the hypothe of H gen & S n-Knudsen (1976) Th delay t low tempera re bet een the ncrea i t ffne s nd th redu t n th rat of ten ion drop m mak the dea pl bl that ttach t of cro -bridge nd forc gene t on t ke pla e two sepa t steps

R f renc

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D 5

The distribution of deoxyglucose during ischemia in the eye

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Accumulation of deoxyglucose in tissue is a useful indicator of metabolic activity especially in nervous tissue (Sokoloff et al 1977). This is so because deoxyglucose accompanies glucose into the cells where it is phosphorylated but not metabolized further. One complication in this method is insufficient O_2 supply. When this occurs the cells are likely to rely on glycolysis whereby large amounts of glucose and consequently deoxyglucose have to enter the cells. The tissue will thus show increased deoxyglucose accumulation while the actual energy turnover is likely to be depressed (Pulsinelly & Duffy 1979).

To investigate the possible usefulness of this phenomenon for detection of discrete regions with marked underperfusion we have tried to map the glucose consumption of the distal part of the optic nerve under conditions of ocular ischemia caused by elevated intraocular pressure, the idea being to determine if and to which extent ischemia results in increased uptake in the region adjacent to perfused tissue. It has been shown previously that in the lamina cribrosa close to its posterior border there is a sharp boundary between perfused and nonperfused regions (Geijer & Bill 1979).

In a monkey which had a normal intraocular pressure in one eye and a pressure causing ocular ischemia in the other C14-deoxyglucose was given i.v. After 40 min the monkey was killed and the eyes removed, frozen and sliced for autoradiography. The autoradiograph showed a strong accumulation of activity in the region of the lamina cribrosa in the ischemic eye. To document details the autoradiographs and slices were studied with the aid of a film scanner (microdensitometer) with 25 µm resolution. Comparison indicated that deoxyglucose accumulation was most marked in the interior of the lamina cribrosa falling off successively in the direction of the interior of the eye. The region of increased accumulation was ca 150–200 µm wide. This pattern seems compatible with conversion to glycolytic metabolism in the ischemic tissue.

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D 6

Effect of dietary calcium (Ca) on secretion of calcitonin (CT) in rats with medullary thyroid carcinoma (MTC)

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Serum Ca is the main regulator of CT secretion. MTC-tumours which secrete CT have preserved the ability to respond to acute serum Ca variations with changes in hormone secretion. We have examined if chronic changes in dietary Ca may alter secretion of CT from rat MCT-tumours transplanted beneath the kidney capsule in young Wag/Rij rats (n=150). The animals were given standard diet 1 week following transplantation. When the experiment started the rats were divided in 3 groups which received varying amounts of Ca: low (0 09% Ca) medium (0 40% Ca) and high (1.60% Ca) for 34 weeks.

After 6 weeks serum Ca was significantly different between the low and high dietary groups: 2.14–0 11 mmol/l and 2 41–0 19 mmol/l (p<0 03) respectively. In contrast, magnesium concentrations showed reciprocal changes: low Ca diet 1.75–0.04 mmol/l and high Ca diet 0 70–0 04 mmol/l (p<0.01). No alterations in serum proteins were observed during the experiment.

Serum levels of CT were measured radioimmunologically using antiserum to human CT directed against the C and N-terminal end of the molecule. Serum CT was monitored from week 12–28 after start of the experiment, but increased steeply (about 4-fold) thereafter. No difference was measured in serum CT between the 3 groups at any time point.

Gastrin is known to stimulate secretion of CT which, on the other hand is able to suppress gastrin release. Serum gastrin was therefore measured radioimmunologically in the 3 groups. The rats on medium and low Ca diet showed lower gastrin levels than the groups on high Ca diet after 6 weeks before changes in CT occured. After 28 weeks the high Ca diet rats with raised serum Ca and gastrin values showed an inhibition in the gastrin levels approaching the other groups. At the time of increased serum CT (week 28–34) the serum gastrin concentrations were low and equal in the 3 groups.

In conclusion, different dietary regimens resulted in corresponding variations in serum Ca and inverse changes in serum magnesium. Since no differences were detected in the serum CT levels between groups on high, medium and low Ca diet, secretion of the hormone and probably growth of tumour cells were not affected by chronic changes in dietary Ca. Increased serum Ca stimulates gastrin secretion and is able to sustain the inhibitory effect of CT on serum gastrin.

D 3

Early effects of cervical sympathetic stimulation on cerebral ocular and cochlear blood flow

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The cerebral blood vessels the extraocular part of the retinal arteries the blood vessels of the optic nerve the choroid and the cochlea are supplied with sympathetic vasomotor nerves The physiological role of these nerves has been debated upon for a long time (see Lassen 1974) During normotension electrical stimulation of the cervical sympathetic chain has little effect on cerebral flow as determined with labelled micro spheres (Alm & Bill 1973) But autoregulatory mechanisms can be expected to modify effects of vasomotor nerve stimulation in many tissues We have tried to reveal an early marked but transient effect of cervical sympathetic stimulation on cerebral retinal and cochlear blood flow

The experiments were performed in anestheti zed rabbits The labelled microsphere method was used to determine regional blood flow after 15 25 sec and 5 min of electrical stimulation of the cervical sympathetic chain At a frequency of 6 Hz there was a 5 % reduction in cerebral blood flow at 15 25 sec and 7 % at 5 min In the choroid plexus the mean reduction was 22 % at 15 25 sec but decreased to 10 % after 5 min In the cerebellum the optic nerve and retina sympathetic stimulation had no appreciable effect on the blood flow In the iris and the cochlea the blood flow reduction was 32 and 25 % respectively on both occations In the choroid vasoconstriction increased with time in the masseter muscle there was a decrease

No indication was thus found in the present experiment for an autoregulatory escape phenomenon in the brain the eye or the cochlea Some escape was noted in the masseter muscle

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D 4

Facial nerve stimulatio and regional blood flow during hypotension

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Morphological evidence for cholinergic nerve supply to the cerebral blood vessels has existed for many years (Chorobski & Penfield 1932) The nerves are supposed to be distributed mainly via the VIIth cranial nerve although the IIIrd IXth and Xth cannot be excluded

A recent study in dogs had indicated a cerebral vasodilation during stimulation of the facial nerve (D'Alecy and Rose 1977) but a microsphere study in our laboratory in normotensive rabbits did not reveal any significant effect (Stjernschantz & Bill 1979) It seemed possible that autoregulatory mechanisms normally abolish effects of vasodilating nerves

In this study a hypotension-model was tested which might reveal normally masked vasodilatory mechanisms

Rabbits (n=14) anesthetized with Iluanison + fentanyl (Hypnorm®) and ketamine-chloride (Ketalar®) were used The facial nerve on one side was exposed at the internal acoustic pore; a lateral or suboccipital approach was used The blood pressure was controlled by bleeding into a reservoir from a femoral artery Tubocurarine (0 5 1 mg/kg b w.) was used for muscle relaxation Labelled 15 μm spheres were used to determine regional blood flow twice during each experiment according to the reference flow method

In one group of animals the first isotope injection was carried out at arterial blood pressures between 35 50 cm H_2O and the second injection during stimulation of the nerve on one side at the same low blood pressure

In another group of animals the nerve on one side was sectioned to stop centrifugal impulses The first isotope was injected at spontaneous blood pressure (85 110 cm H O) and the second i jection after a pressure decrease to 35-50 cm H_2O

The results indicate a strong vasodilatory effect of facial nerve stimulation in the mandibular gland in the mucous membrane of the tongue in the choroid and the ciliary processes but no effect in the brain Sectioning of the nerve did not change the blood flow

Thus in rabbits under general anesthesia and arterial hypotension caused by bleeding cerebral vasodilation is not likely to be due to facial nerve activity

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D 9

proportion between local uptake of 15µm microspheres and local renal blood flow? Model experiments

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Measurements of blood flow distribution in the kidney performed with microsphere (MS) assign greater percentage f flow t the outer parts of the cortex than do diffus ible indicators under control conditions (Clausen et 1 1979) The discrepancy could be due to kimming f MS t the origin of the afferent arteri les ca ing rise in MS concentration in the inter lobular arteries towards outer cortex Disappearance of the difference during vasodilation (Clausen et 1 1979) might suggest the diameter of th interlobular artery as regulating factor To deter mine the importance of diameter chang we have performed model experiments using glass lit model (Palmer 1965 Øfjord et al 1979) The main channel represents an interlobular artery and 25µm wide id branch an afferent arteriol In the model we have varied the width or diameter" f the interlobular artery" Citrated platelet free human blood (Hct 40) containing radiolabelled MS (10 000/ml) was run through the lit model t constant inflow velocity From the branching lit fraction of 2 to 50% f total flow wa taken out by varying th outlet esistance Main channel width trongly influenced the degree of kimming U ing a 64µm wid main channel and 5% b anch flow the concentration of red blood cell leaving the branch wa 75% f that f inflowing blood By decrea ing the main channel to 50µm the red cell concentr tion was reduced to 55% The concentration of 15µm MS was cor espondingly educed from 35 to 5% The results show that relatively small changes in main channel width cause dramatic changes in the extent of kimming Thi supports the idea that hanges in interlobular artery diameter cause intrarenal edistribution of MS relative to glomerular blood flow

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D 10

Lipolysis induced y vasodilatati i hum adipose tissue during rci ...

... ...

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It has p eviou ly b hown that adipo tissue blood flow incr ase i subcutaneous and p irenal dip ti sue du i g prolonged xercise i an (1 2) T investigat whetha thi sodil tation i secondary to the enhanced lipoly i whether it i dire t result f increased stimulation f lar b ta-receptors the f llowing ime ta re car ried out

... young he lthy al p rf rmed moderate to heavy rci on bicycl rgometer for 4 hour und 4 diff t ndi tions control conditions, during ni ti ... acid blockade f lip lysi du ing ... l block de f beta-receptors and ... tinou gl cose i f ion I ch ... subcutaneou ATBF wa me sured ... the modifi d 133X wa h-o t method ... Blood was d aw every hour f det ... f gluco glyc rol and f ... acids

... ontrol xpe i t and i the ... xp iment ATBF rose 3 ti ... initi l re ti g ontrol ... I the nicoti i cid pe ime t no ... incre wa found and i the rop ... xp ime t ly an insignifi ant inc ase was f und Pl ama glycerol ind 4 f ld i th co trol xperime t comp red t the re ting lue, i the gluco xp im t it ro 3 f ld i th prop anol l xp i ts 2 f ld whil no increase could be demo t t d i th nicoti i acid xp iment indi ti g that lip ly i wa ff tively bl ck d i th xp im ts

I nicotini acid xpe im t imilar t the pre t the ci cul ting l l f t holamin ha been shown to be highe than during co trol conditi ns (4), consequ tly an incre i flow qual t or ve gre te than that found i the control xpe i t would b xp t d if ... cular beta-recept rs we f ny i po tan... f the odil t tion It i ncl ded that the asodil tati i adipo ti sue du ing rcise i induced ond ry t enhanced lip ly i nd t by direct ti mul tion f ... scular b ta-re ept

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D 7

Storage of frogs at low temperature for prolonged periods induces major ultra-structural alterations of the mesenteric capillaries

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The ultrastructure and permeability of single mesenteric capillaries in the frog (Rana temporaria) are studied at our laboratory Prior to the experiments the animals are kept at 4°C for varying periods of time During storage at this low temperature the animals are starved and undergo seasonal cycles which imply major physiological alterations (Barker Jørgensen et al 1978) The possibility that this cold storage may influence the microvasculature was investigated in a quantitative ultrastructural study Mesenteric capillaries from two s ts of frogs captured in late summer were compared One group of ani mal was kept at 4°C for less than a week (short-term frogs) and the other group at 4°C for 6 8 months (long term frogs)

The mesenteries were exposed and initially fixed by superfusion with a buffered solution of aldehyd s Randomly sampled cross sections of 31 capillaries from short-term frogs and 51 capillaries from long term frogs were included in the study Analysis of the electron micrographs was performed at a final magnification of 61600 x

The diameter of the capillaries the average thickness of the endothelial cells the depth of the interendothelial clefts and the appearance of the endothelial junctions were similar in the two groups of animals However the volume density of the endothelial vesicles was 12 8% in long term frogs versus 6 4% in short term frogs The length of the interendothelial clefts per µm capillary wall was 2120 Å/µm² in long term frogs and 1200 Å/µm in short term frogs For both parameters there was a significant difference ($p<0\ 005$) between the two groups In 6 out of the 31 capillary cross sections from the short term frogs interendothelial gaps 200 500 nm wide were observed No gaps were encountered in all 51 capillary cross sections from the long term frogs

We conclude that storage of frogs at low temperature for long periods of time induces major ultrastructural alterations of the capillaries We suggest that the stage of the animals within the seasonal cycles and their nutritional condition should be well defined in physiological investigations of frog capillaries

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Inhibition of drug metabolizing enzymes by chlorinated phenol in vitro

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Chlorinated phenols when added to in vitro incubation were shown to be strong inhibitors of enzymes catalyzing hydroxylation or glucuronidation reactions hepatic microsomes of the rat. 13 uM of pentachlorophenol (PCP) in the incubation mixture decreased the activities of arylhydrocarbon hydroxylase and ethoxycoumarin deethylase to ... of the control value 10 uM of PCP decreased the glucuronidation f 2-aminophenol by ..., whereas 1 uM of PCP was sufficient to reduce the glucuronidation of 4-methylumbelliferone to ... of the ... activity

Hydroxylation and glucuronidation reactions were inhibited by tetra- tri and dichlorophenols ... The inhibitory potency was dependent on the position of chlorine substituents At strongest the inhibition of drug metabolizing enzymes by tetra- and trichlorophenol was of the same order of magnitude tha the inhibition by PCP

Phenol and chlorinated benzenes (di to hexachlorobenzene) themselves were not inhibitors of drug metabolizing enzymes Thus the inhibition caused by chlorinated phenols i a combined effect of both the phenol group and the chlorine substituents of the molecule

Pentachloroaniline lightly inhibited hydroxylation reactions whereas pentachlorotoluene had no ffect on the activities of drug metabolizing enzymes i itro

Epoxide hydratase and glutathione S transferase activities were not affected by chlorinated phenol in vitro

Grants The Finnish Academy of Sciences
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D 14

Effect of 7-oxa-13-prostynoic acid prostaglandin induced LH release in ...

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Prostaglandins (PG) seem to play role in the neuroendocrine regulation f pituitary gonadotropin secretion by stimulating the release f luteinizing hormone releasing hormone(LHRH) from the hypothalamus This activity f PG may depend on thei capacity to activate specific binding ites in the brain The present study wa undertaken to investigate thi hypothesis by utilizing th PG-analogue 7-oxa 13-prostynoic acid (7-OPA) which is belived to interfere with receptorbinding f PG
The analogue wa infused into the reventricular sys em f anesthetized male rats ... in p i to intraventricular administration f PGE_2, PGF_2 LHRH and ... concentration f LH in arterial plasma as determined during 90 mi period Infusion f 7-OPA alone had no ffect on the release f LH However when 44 132 g of 7-OPA was infused in combination with 2 g f PGE_2 the LH releasing activity f PGE_2 increased with increasing priming doses f 7-OPA At 60-90 mi the LH concentration was 2 3 fold higher in animals pretreated with 88 132 g f 7-OPA than in control rats receiving the 7-OPA diluent The minimal effective dose of PGE_2 (1-o 5 g) decreased fter pretreatment of the animals with 7-OPA nd formerly subthreshold doses (o ol g) was found t become an effective stimulus fo LH release whe administered subsequent to infusion f 132 g 7-OPA When 7-OPA (132 g) was infused prior to PGE_2 th dose-response curve f PGE_2 (ove the rang o ol 2o g) we displaced to the left without chang in the maximal respons
Administration f 132 g of 7-OPA before an intraventricular infusion of 5o ng LHRH did not alter the stimulatory effect of thi hormone on the release f LH indicating that 7-OPA acts t supr pituitary ite to potentiate th LH releasing activity f PGE_2
In co trast to its potentiating ffect n PGE_2 induced release f LH 7-OPA did not influence the stimulatory action f $PGF_{2\alpha}$ (2 g) on the secretion f LH
Th present findings are consistent with the view that PGE_2 activates specific binding ite in the brain as an event prior to stimulation f LHRH (and LH) release The lack f effect f 7-OPA o $PGF_{2\alpha}$ induced LH release suggests the existence f separate binding ite f PGE_2 and $PGF_{2\alpha}$

D 11

The effect of vanadate on liver hemodynamics and bile production of the perfused rat liver

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Vanadate has recently been found to be a potent inhibitor of Na-K ATPase (Josephson & Cantley 1977) and in experiments on cats vanadate also appeared to be a potent constrictor of renal blood vessels (Larsen et al 1979) In the present experiments the effect of vanadate on liver homodynamics and bile production was examined in 11 perfused rat livers Furthermore the effect of vanadate on the Na K ATPase activity in isolated canaliculi-enriched liver plasma membranes (LPM) was examined

Vanadate caused a dose dependent fall in portal blood flow and conductance which was reduced 11 29 and 50% at vanadate concentrations of 9 18 and 50 µM respectively At vanadate concentrations of 9 and 18 µM the hepatic oxygen consumption and bile flow remained constant but at 50 µM oxygen consumption decreased 20% and bile flow 28% The biliary concentrations of van - date were identical with plasma vanadate concentrations Na-K ATPase activity in LPM was inhibited 50% by 0 25 µM vanadate

The present results demonstrate that vanadate is a potent constrictor of liver vasculature at concentrations which do not affect oxygen uptake or bile production The observed decrease in bile production at high concentrations may be secondary to hypoxia and a specific effect of vanadate on bile production which to some tent may be dependent on the activity of Na K ATPase could not be demonstrated

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D 12

The distribution and function of the sympathetic innervation I liver and endocrine pancreas

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Sympathetic noradrenergic nerves with their well known cardiovascular effects have recently been found to influence several metabolic and hormone-releasing processes Thus experiments on animals have shown a considerable influence of sympathetic stimulation on liver function Stimulation of the liver branches of the splanchnic nerve are associ ted with variations in the rate at which glucose is released from the liver (Edwards 1972) In addition it has been demonstrated that stimulation of sympathetic pancreatic nerves evokes a pronounced liberation of glucagon as well as an inhibition of insulin release from the endocrine pancreas

We have recently investigated the morphology of the sympathetic nerves to the liver and endocri e pancreas in man With the Falck Hillarp histofluorescence techniques for visualization of catecholamines on the cellular level it was demonstrated that the human liver parenchyma as well as the human islets receive an abundant supply of nerve fibers displaying the typical catecholamine fluorescence (Nobin et al 1978) Quantitative determinations revealed that the noradrenaline content of the human liver is about 1 µg/g wet weight which leaves little doubt that these liver nerves contain noradrenaline Electron microscopy confirmed that the adrenergi nerve terminals contacted the individual hepatocytes When peroperatively stimulating the sympathetic liver nerves clearcut increase in the arterial plasma glucose concentration was achieved reaching a significant peak increase of about 2 mM above the control level at the end of 10 timulation

Regarding the function of the direct adrenergic fibers to the islets of Langerhans we have shown that they are involved i reflex hyperglucagonemia and hypoinsulinemia in respons to a blood pressure drop and also that they participate in the regulation of glucagon and insulin during hemorrhage and hemorrhagic shock (Järhult t al 1979)

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D 13

Inhib tion f drug metabolizing enzymes by chlorinated phenol i vitro

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Turku Fi land

Chlorinated phenols when added to vitro incubations were shown to be trong nh bitors of enzymes catalyzing hydroxylation or glucuronidati on reactions n hepatic microsomes of the rat 100 uM of pentachlorophenol (PCP) i the incubati on mi ture decreased the activities of arylhydrocarbon hydroxylas and ethoxycoumari deethyl se to 48 % f the control lue 10 uM of PCP decreased the glucuronidation f 2-aminophenol by 60 % whereas 1 uM f PCP was suffi ent to reduce the glucuron dation of 4-methylumbelliferone to 47 % of the nhibited activity

Hydroxyl tion nd glucuron dation reactions were nhib ted by tetra- tri nd dichlorophenols too The nh bitory potency was dependent on the pos tion of chlorine subst tuents At trongest the inh b tion of drug metabol zing enzymes by tetra and trichlorophenol was f the same order of magnitude than the inhibition by PCP

Phenol and chlorinated benzenes (di to hexachlorobenzene) themselves were not inhibitors of drug metaboli ng enzymes Thus the inhibition caused by chlorinated phenols is combined effect of both the phenol group and the chlorine substituents of the molecul

Pentachloroaniline lightly inhibited hydroxyl tion reactions whereas pentachlorotoluene had no effect on the activities of drug metaboli ing enzymes i vitro

Epoxide hydratase and glutathione S-transferase activities were not ffected by chlorinated phenols i vitro

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D 14

Effect f 7-oxa-13 prostynoic acid prostaglandi induced LH elease i mal ts

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Prostaglandins (PG) eem to play ro- l in the neuroendocrine egulation of pituitary gonadotropin ecretion by sti mulating the release f l t ini ing ho mone leasing hormone(LHRH) from the hypothalamus Thi acti ity f PG' may depend on their capacity to tivat specific binding ite in the brain The present tudy we und rtaken to investi gate this hypothesis by tilizing the PG-analogue 7-oxa 13-prostynoi id (7-OPA) which i belived to interf re with receptorbinding of PG
The nalogue was infused into the cerebroventricul sys em f naesthetized mal rats 3 min p ior to i traventricular administ tion f PGE_2 PGF_2 LHRH and the concent tion f LH in art rial plasma was det rmined during 90 min period Inf ion f 7-OPA lone had no ffect on the leas f LH Howeve when 44 132 g of 7-OPA wa infused i combination ith 2 g f PGE_2 the LH relea ing acti i ty f PGE_2 increased with increasing priming dose f 7-OPA At 60-90 mi the LH concentr tion was 2 3 f ld higher in ani mal pretre ted with 44-132 g of 7-OPA than in control rats recei ing the 7-OPA dil t The minimal ffective dos f PG E_2 (1-o 5 g) decr ased aft pr treatment f the animals with 7-OPA nd a formerly subthreshold dose (ol g) wa found to become an effective stimulus f LH e-lease when administered subsequent to infusion f 132 g 7-OPA When 7-OPA (132 g) wa infused prior to PGE_2 the dose- esponse curve for PGE_2 (ove the rang 1 2o g) wa di placed to the left without chang in the maximal esponse
Administr tion of 132 g of 7-OPA bef re an intraventricular infusion f 50 ng LHRH did not lte th timulatory ffect of this hormone on the relea f LH indi t ing that 7-OPA acts t uprapituitary ite to potentiate the LH leasing cti vity f PGE_2
In cont ast to its potentiating effect on PGE_2 induced leas f LH 7-OPA did not infl ence the timulatory tion f $PGF_{2\alpha}$ (2 g) on the secretion f LH
The present findings re consi tent with the iew that PGE_2 ctivates specifi binding ite in the b in as event pri or to stimul tion of LHRH (nd LH) rel ase The lack of ffect f 7-OPA $PGF_{2\alpha}$ induced LH releas uggests the existence f separat binding ites f PGE_2 and PGF_2

D 15

Synaptic long lasting potentiation in hippocampus its relation to afferent fibre threshold and t tanization strength

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Guinea pig hippocampal slices in vitro (Skrede & Westgaard 1971) were used to study long-lasting pot ntiation (LLP) of synaptic transmission to CA1 pyramidal cells Since previous studi s employing two sep rate inputs to the pyramidal cells (Andersen et al 1977 Lynch et al 1977) hav shown LLP to be specific for the t tanized path, we now wished to det rmine whether the specificity holds also for different fibres within the same path

El ctric stimulation was delivered to the fibr s in stratum ori ns at a low r petition rate interrupted by short periods of t tanic stimulation The slope of the relation bet een presynaptic volley and fi ld EPSP was used to estimate th LLP contributed by oriens fibr s with different thresholds for electric stimulation

In 6 out of 12 slices there was a considerable contribution to the potenti tion from not only the fibres recruited below tetanization strength, but also from the higher thr shold fibres This finding seems to indicate that LLP is not tir ly specific to th indi idual fibres s bjected to t tanization

Alternative interpretations f the esults r possible For instance the high threshold fibres would certainly contribute to LLP if they took part in tetanization, due to a te rary decrease in threshold This seems unlik however since no increas in the presynapti volley amplitude as seen duri g the teton s Another possibility would be that the activa tion of potentiated synaps s could mod l t transmission at those synapses hich have no been potenti ted

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D 16

The ff t f controll d physi l t l ing on lipid met bolism in men A l ngit dinal t dy

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The Rehabilitation Resea ch Ce t   f th Soci l Insur c I stit tion T ku nd Dep tment f Physi logy Uni   sity f T ku Fi land

Phy ical acti ity ha bee sugge t d t have beneficial effect on lipid metabolism Mo t studi s concerning the effect f phy ical t l ing on plasma lipids (eg L hton   & Vilk i 1978) or postheparl plasma (PHP) and dipos ti ue lipoprot in lipas (LPL) (eg Nikkilä t l 1978 P ltonen t al 1979) have been ca l d out by meas ring thes paramet rs i group   f s bject differing in their phy ical   ti ity The i of the pre ent st dy was t i   tig t both plasma lipids and PHP and dipos ti ue LPL longit dinally i subj ct   ndergoing   ontroll d physical t ai i g program

The traini g group con isted f 20 previou ly untrained men who t ained nd   control f 15 we ks at l ast three hours a week t i ing consisting mainly f running or ki g   Du ing the same period   control group of even me were ask d to maintain their normal physical sonal a i tions acti ity t control possible s At the beginning of in the meas red parameters At the beginning of the training period the groups did not diff   i r l tion t ag   relati   body weight r physical fitness

Du ing the test pe iod physical fit ess i e   d ig ificantly (P 0 001) i the t i ing group   calculated by the pai ed t t t whe no hange occu ed i the ontrol group   i the t i ing group i   PHP LPL (P 0 001) adipos ti ue LPL (P 0 005) HDL hol t rol (P 0 005) nd HDL/t t l chol terol   tio (P 0 001) i re sed where   t t l hol t rol (P 0 10) LDL chol st rol (e timated by   lcul tion) (P 0 02) and i   ii (P 0 05) dec e s d during the t i ing No ignifi ant hange we obs rved i thes pa amete   i the ontrol group The rel ti   hange i HDL/t t l hol t   i   ti co   l ted po iti ely with the absol t change i phy ical fitne   (P 0 05) and ith the i ti ve hang i PHP LPL   ti ity (P   0 05)

Ou   lt   i good ag eement ith the ii ro   ectional t di   and i ly ndi t that mod t t i ing prog am f fift en week i enough t   compli h benefi i l hang   i lipid met boli

Suppo t d by Y jö J h son Foundation Fi land

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The effect of adrenergic blocking agents on the splanchnic release of 5 HT from enterochromaffin cells in the cat

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Cat splanchnic nerve were timulated lectri cally (8 10 V; 2 m ; 8 10 Hz; 3 5 min) at the preganglionic level in fferent direction in chlora lose anaesthetized ats (n=6) with the adrenals lig ted bilaterally A ignifi ant decrease (p< 0 05) of the intra ellula se otoni (5 HT) levels in populations (n20) of individual enterochromaffin ll (EC) in the mucosa from 3 different level of the small intestine (duodenum midjejunum, terminal ileum) wa obtained. The intracellula 5 HT levels befor and afte timulation were studied cytofluorometrically in biopsies treated according to the Falck Hillarp technique (Ahlman et al 1976)

One group of cat (n=5) wa pretreated with propranolol (2mg/kg) prior to stimulation This prevented the decrease in fluorescence intensity ffectively Two othe groups of cats (n=4 ach) were pre re ted with phenoxybenzamine (5 mg/kg) or phent lamine (5 mg/kg) re pectively whi h also blocked the decre se in fluor cence intens y xc pt in one animal In few biopsi there wa an incre sed fluore cenc intensity afte nerve stimulation in ats give propranol l and phentolamine respectively eve though the drug themselve had no intrin ic ffects on the fluor ace inten ity in control animals

I I oncluded that ff nt lectrical timula tion of the splanchal nerve can cause rele f 5 HT from gut EC by an adrenergi mechanism which is good accordanc with ea lie in vitro tudie (Pette on t al 1979) and lectron mi ros opy finding (Newson t al 1979)

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D 18

Comparison between the ffects of diethyl-stil bestrol on uterine, cardiac and skeletal muscles

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Diethyl-stilboestrol (DES) one f the most potent estrogens exerts marked effects on contractile acti ity of uterine cardiac and skeletal muscles DES in icromola concentrations has strong relaxing ffect on uterine smooth muscle (Batra & Bengtsson 1978) and t also exerts negative inotropic ffect on cardiac muscle By contrast DES has powerf l potentiating ffect on the t tch response f skeletal muscle (Khan 1979) Studies on skeletal and cardiac muscles show that DES, in these low concentrations does not ffect the resting and action potenti ls On the other hand DES has been shown to inhib t the cal ium influx through the lemma membrane (Batra & Bengtsson 1978) as also the rate of resequestration of cal ium by the isolated sarcoplasmic reticulum (Khan 1978 unpublished data The result support the idea that uterine and cardiac muscles predominantly depend on ex racell la cal ium influx for their cont act l acti An inhib tory ffect f DES on the cal um influx i thus likely t be responsibl for the decrease in contractile act it in these muscles The contractil response f skelet l muscl is on the ther hand not imed tely dependent on calcium influx rom the external medium (Andersson & Edman 1974) In thi muscl calcium is released from and resequest red by the sarcoplasmic reticulum (SR) forming virtually losed cycle The inhib tory effect by DES on the SR calcium pump may explain the potentiating ffect on the t tch response f skeletal muscl fibres This is supported by the finding that DES not only increases the twitch amplitude but lso prolongs the relaxation phase in skeletal muscl The relaxation of cardiac muscle is on the othe hand not gnificantly ffected by DES This could be due to lower sensit vity f SR in cardiac muscle to DES or lternatively that limination f calcium in cardiac muscl is largely accomplished by another system than SR

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28 A

D 19

The metabolism of activator calcium in mammalian myocard um ev luated from an analys f trength-int rval rel tions

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The following model f r the metabolism f activa tor calcium in the cardiac cell (Wohlf rt 1979) has been used i order t explain the inotropic change that is *produced by altering the stimula*tion interval Calcium i released from a st re Y into the myofibrill r space during the action potential The mechanical response i thought to be directly rel ted to the amount f calcium *released Relaxation occur as calcium is with*drawn from the contractile system A portion f th e calcium (approximately 0 3 see below) is taken p by a tore X and i added to the calcium that has entered the cell (and store X) during the action potential *The* calcium contained i st re X i finally transported t the releasable tore Y The complet replenishment f store Y fter a release (at 37°C) requires time period f 0 8 s The amount f cal ium in Y decreases slowly due t continuous *leakage* The calcium inflow during the action potential i inversely rel ted t the calcium contents f a st re Z R lease occurs from Z during the action potenti l and the replenishment i a time dependent process that occurs between action potenti l The rate of replenishment of Z i proportional to the amount f c lcium in tores X and Y The i flow of calcium during the ction potential will therefore be an inverse function of the inotropic state f the muscle

The model explains why OCR optimum contractile response (Edman & Jóhannsson 1976) is obtained 0 8 s fter a preceding stimulus The increase in OCR ft r an xtra stimulus (postextrasyst lic potenti tion) i due to: (1) a rel tively small *release of calcium from st re Y by the extra sti*mulus and therefore small loss of calcium out of the cell and (2) an enhanced transport of calcium int the cell during the extra action potential (low content of calcium in Z) Postextrasystolic potentiation decays slowly *if the muscle is left* unstimulated due t the steady leakage of calcium from st re Y However the potenti ted stat decays more rapidly if the muscl is timulated as a relatively large fra tion (approximat ly 0.7) of the released calcium leaves the cell The model furthermore xplains the biphasic rise in contractil f rce in response t increased stimulation frequency and lso the enhancement of OCR that is seen after period of high stimu-l tion frequency (post timulation potentiation) The model predicts that OCR ill increase ith the stimul tion frequency whereas the teady-state response will rise t ma imum value and then decline

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D 20

Gonadotropin i teraction on cycl AMP forma tion in sol ted theca cell from preovul t ry rat f ll les

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Preovulatory f lli les were sol ted from t 2 days afte treatment ith a ngl injection f PMSG (Herlitz t al 1976) From the f llicles theca cell were mechanically isol *ted The cells* were subsequently incubated f 1 h the pre sence r absence of LH or FSH and the accumu l tion f cycli AMP i t sue plus medium was det rmined In ce tain experiments the rats were injected with *LH FSH o saline 2 h p* t sacrifice

When the cell were isolated from r t k lled before the endogenous gonadotrop surge FSH i vitro did not stimulat cycli AMP f rmat on will LH caused small but significant timu lation When isol ted in the evening ft the endogenous LH-FSH peak the theca cell re sponded ith marked increase in cAMP f rmat on when exposed to LH in itro whereas FSH in itro had no ffect

When the cells were isol ted i the morning-before the LH-FSH peak and the r t were pre treated ith ingl i p injection of *FSH 2 h* prior to sacrifice the cell responded ith marked increase in cyclic AMP formation t subsequent in itro xposition to LH Thus pre-tre tment ith FSH which per se does not i fluence AMP f rmation made the cell more sensi *t ve t LH*

These result indicat that the FSH component of the endogenous gonadotrop surge increases the sensit ty of the theca cell t *LH i* in vo expos tion t FSH seems to be essenti l *f r the developement f responsiveness* of the cycl c AMP ystem to LH n the preovul tory theca cell As demonstr ted as li r for iso-l ted granulosa cell (Richards J S 1975)*FSH* seems t be necess ry f r development of LH recept rs *the theca cell*

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contraction dynamics i smooth muscl t diffe ent
odes of act vation.

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The mode of activation has been observed t influence the force-velocity rel tion i smooth muscl (e g Murphy 1976) but systemati comparisons have not been reported We have determined series l ast ti and force-velocity properties i individual muscles under stimulation by AC current and K^+-high medium respectively The expts were perf rmed on preparations f two different kinds f smooth muscle, the rabbit urinary bladder and the rat portal vein. Each preparation was first stimulated by repeated 2-5 mi exposures to K^+-high solution (100 mM K^+ substituted for equal amount f Na^+) then by AC-stimulations (30-60 H 2 5-3 V) each lasting 5-10 The AC stimulation parameters were adjusted to give the same isometri tension as the K^+-contractures One instant qui k releas was made at the peak f each contraction with ftermed varied in random fashion Records were stored on magneti tape and subsequently analyzed in computer (Hellstrand and Johansson 1979) The amount of lasti recoil was calculated as was the shortening velocity at 100 ms after relea The force-extension relation of the l sti lement was determined by fit to the equation

$$P \; A \; exp(k \; \Delta L/L) \; B \qquad (1)$$

The force-velocity data were analyzed according to the Hill equation

$$V(P \; a) \; b(P_0 \; P) \qquad (2)$$

P_0 and V_{max} were determined as the intersections of the force-velocity hyperbol with the f rce and velocity es respectively

The esults re summarized in the tabl showing P_0 k and V_{max} unde the two timulation condi t on Mean SE shown *=p<0 05 **=p<0 01 by Student t-test for paired data

		P_0 mN/mm^2	k [cf. eqn(1)]	V_{max} lengths/		
Bladde	AC	33 4	51 6	0.39	0 06	6
	K^+	33 4	54 3	0 26*	0 03	
Port l vei	AC	24 6	32 2	0 48	0 09	7
	K	21 4	37 5	0.32**	0 06	

Thus at i ile P_0 the stiffness of the lasti lement i the same for both timul tion conditions whereas V_{max} i ignificantly lower i K -contractures both i bladder and portal vei

The data give no i formation as t where i th ex itation-contraction sequenc the observed diff rence i V_{max} arises It i possibl that diff rent metabol condit ons exi t or that ltrastructural changes i K^+-high medium as have been reported by Jones et l (1973) re of relevenc

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Extracellul r H and K concentrati ns in b in cortex following t minutes f cerebr l ischemia

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The linical estit tion f te aft 10 minutes f cereb l ischemi i seve ly imp i ed by glucose p et eatme t (Siemkowicz & Hansen 1978) Bec use low pH was suggested t be th cause f cellular damage during ischemi we measured b ain extracellular pH and $[K^+]$ i normogly emic and hyperglycemi ts The pota ium measurement was included to provide an i dex f the eve ity f ischemia

Ischemi was induced by infl tion f r neumati cuff round the neck and reduction of MABP by emoval f blood (Siemkowicz & Hansen 1978) Aft craniectomy f r l f pari l bone $[H^+]$ and $[K^+]$ were measured i the cortex by ion-selective icroelectrodes

Plasma glucose as raised p i to the onset t ischemi by glucose admini tration i p

f normoglycemi rats plasma glucose was 8 1 mM (SD) i hyperglycemi 28 2 mM Ext acellul pH began to f ll imedi t ly f llowing th onset f ischemi decreasing from 7 35 0 05 t 6 55 0 20 i the normoglycemi rats and from 7 10 0 06 t 6 12 0 06 i th hyperglycemi group Extracellula $[K^+]$ displayed the low and rapid ph es of increase i both groups

Th dur tion of th low phase averaged 1 6 mi i th normoglycemi te and 3 4 min i the hyperglycemi ts

In both groups $[H^+]$ and $[K^+]$ returned to normal fte ischemi Normali tion f $[K^+]$ was compl te after 3 5 0 5 mi in th normoglycemi rats and 4 1 0 6 min i the hyperglycemi ts (ns) Ext cellular pH was normal fter 24 4 mi in the normoglycemi and 29 6 mi (ns) i th hyperglycemi rats

The results indicate that i ni homeost i f hydrogen and potas ium i th extracell lar space of the cerebral cortex i estored fter t minutes f cereb al ischemia despit marked diff rences f extrac ll lar pH during ischemi and f th l t impaired linical estit tion

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D 23

The cardiac output during rebre thing and t teady tate work A comparison f two modifica tions f non in asive t chnique

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A non i asive method f r measuri g cardi output (Q_I) by det rmi ing the cl rance of an ine t olubl gas (cetylene) from losed rebre thing y tem by help f mass-spectromete has rece tly bee de cribed (Sackne t l 1975 Tri bwasse t al 1977) Earli howeve many determina tions of cardi output i merci physi logy were based on the cetyl ne-method as described by Grollman 1929 (Q_{II})

Th aim f the prese t tudy was to compar the tw methods t re t and during re ise inclusive maximal mercise test

8 human subj cts were tudied t two occasion The results howed high reproducability and an xpe ted i cr ase ith i cr ased oxygen uptak (0 91-0 95) Q_I value were ignifi antly highe than Q_{II} value he the xygen uptak was below 2 l mi l pe i lly pronounced t re ting conditions where Q_I alue were almost 100% highe than Q_{II} lue By including measurements f the oxyge uptak (VO_2) and heart t it was l arly hown that the cent l circul tion i ccel ted during rebre thing l ading to i creased Q_I lues as Q_I indicat the cardiac output during rebre thing whil Q_{II} records the ardi output t teady tate bef re the rebre thing tarts Co re ting Q_I by help f the ti VO_2(teady tate)/ VO_2(rebre thing) thus almost liminates the diffe rence between the re lts The remaining dive gences can mai ly be xplained by methodological rro as method I include the pulmonary ti sue volume i which acetylene i solved i iti lly hil thi volume has bee negl cted i method II

The present tudy thus emphasize that ardia outp t measured during rebre thing should be corrected to t ady-state ci cumstances i order to get prope results t re t and during low mercise l vel

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D 24

Comparison of cat electroradiculogr phy r corded from c taneous intramuscul r nd intradural level

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Examinations of the lumbosacr l root potentials evoked by peripheral nerve stimulation have been performed e g with cats (Happel 1975) and also with humans (Ertekin 1978)

In thi report we compare conductivity values of lumbosacral roots by r cording the potentials with electrodes placed on the skin surface intramuscularily and int radurally We found that on th root level the seventh lumbar segment different recordings give significantly diff t conductivity values depending on the el c trode locations

The sciatic nerve of nine cats was ti mulated both limbs separately sup amaxi mally with needle electrodes on th level of popliteal fossa The responses were recorded with surface cup electrodes and intramuscular needle electrodes bipolarily The electrodes were placed between the seventh lumbar and first sacral spine with the reference electrode between the sixth and seventh lumbar spine After dissoction of the paraspinal muscles intradural recordings were taken from the same level

The latency was measured to the beginning of the first negative deflection and to the peak of the negative deflection (positivity downwards) The negative deflection of the potential was defined as the maximum ampli tude of the response

Means of mea ured values Latency to the beginning of response with surface techni que w s 1 0 ms and to peak of negative de flection 1 6 ms Maximum amplitude was 55 V Correspondingly in intramuscular recordings: 1 2 ms 1 7 ms and 75 uV Correspondingly in intrad ral recordings: 1 3 ms 1 8 ms and 350 uV The corresponding latency values to the beginning of response were significantly different between skin and intradural recordings ($p \leq 0.01$) and be tween kin and intramuscular recording ($p \leq 0.05$)

We found ignificant difference in la te cio hown by vario s recording techni ques at lumbosacral root lev l not only on the uppe cord levels as Happel (1975) claims It seems to s advisable to take count of diff re t recording techniques when lumbo cr l oot conduction time are estimated

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I t rsti i l fluid p in sub cu i and sk t l l me ur d with micropuncture

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Insti t f hy i logy U i i y f Berge Norw y

Inte titial fl id pr (IFP) i ub- antia ad k l tal mus l h be me ured ith needl tech ique impl t d cap l and l k ics ll the thod imply measida abl ume cha g i ti ue struc we decided me IF through sharpe d gl i pipett In Nembutal an th i th pl d supine on he ing pad th hindlimb rotated ou ards and fi d i th an l j int I thi posi i pipe f 1 2 5 µm wi t tr d d int ubc i th ugh inta t ski on the medi l pe t f h thigh unde the guidan f t ee- microscope Th l pipe t an d Wiederhielm's (cf Wiederhielm et al 1964) high llows meas eme ith dding mov- ing any fluid f om th i itium I sk l tal mus l th pun i mad through s mm l ng i i i i he ki the medi l spe t f the thigh Th p ssure were p d b 1) f db k f ce ld be hang d itho l ti the p ur d 2) i t amo f fluid could b i hdr wn i the gl ary ll ry While th i i we f ti fi d immedi ly pon pun t th pipett owetime h d to be pulled lightly b k s t bing omp i g h ti ue hang d th p t eg tive nd po iti val pe i ly All pt s me me t mad ith t i ibl di to i f th ki

ll me me ts i ub ti w ub tha ph i hil th p i k l tal us l abov well below th me ph i p ur M ou ese i 6 t g ve pre ur in when i f 1 41 (SD 0 50 33) mm Hg k l tal us l me ur me t i 16 ts g me pre f 0 12 (SD 1 10 35) mm Hg s li i fu- i in phr omi d t i d th p i be h ti

Th p ur btai d ith mi puncture g ll ith the bt in d ith th wick i dl hniqu i ubcuti (R d R d ukland 1977) an k l t l l (kl d Joh 1974) but nsid ably high than th in h smie- lly impl ted paul (G y 1963) I lusi th p method f ur me f F i pid d p ti lly wmati

f

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D 26

Distribution f ouabai binding site along the dog nephron

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Sodium reabsorption in the thick ascend ing limb f Henl loop (TAL) seems to be carried out by Na K ATPase which i present in high concentr tion in the outa medull Th function f N K ATP se in sodium reab- sorption in the proximal tubules (PT) is less lear and in terms f dry tissue weight the acti ity f the enzyme i low in PT compa ed to TAL and the distal tubules (DT) However PT are long and tortuous and the cell are large with high protein content Hence the total amoun of Na K ATPase in PT might exceed the amount in other nephron segment and the enzyme con centration in relation to tubul length might app oach that f TAL To examine this hypothesi the rel tive concentration and distribution f ouabain binding ites wer estimated by quantitative autoradiography (ARG) in dog kidneys

^{3}H-ouabai 120 nmol/kg with specific acti ity f 50 Ci/mol wa injected int a renal artery f an anae thetized dog By thi injection technique more than 80% f the Na K ATPa activity i extinguished and ouabain i exclusively bound t the enzyme (Sej r ted 1979) in at ischio- metri tio of 1 1 Sections f r ARG we obtained from biopsie treated with gl tar- ldehyde and OsO4 dehydrated with ethanol and embedded in Epon About 11% of the activity was lost during prepar tion

The umber of grains par µ^2 of tubular walls was identical in PT and DT but higher by factor f 2 7 in TAL Neve theless due to difference i tubular wall thicknes the number of grain pe cros section did not diff in PT and TAL but wa more than 40% lower in DT Thus the numbe of ouabain binding ites pe tubular mm i identi l in PT and TAL About 85% f the corti l grains were loc li ed over PT Because 77% f all renal ouabain binding ites are found in the cortex (Sejersted 1979) PT contain about 65% (0 85 0 77 100%) f the total renal amount of Na K ATPase and DT about 12%

Compared t transcell lar sodium eab- sorption rates Na K ATPase seem to be present in gre t excess in PT bec se as much 60 70% of the absorbed sodium passes by the paracellular route (Mathisen et l 1976) In TAL and DT however maximal sodium reabsorption r te could be lose to the maximal pumping apa ity of Na K-ATPase

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D 27

A telemetry study on deep body temperature of the Willow Tit (Parus montanus)

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Temperature measurements on Black capped Chickadee (Parus atricapillus) and the closely related Willow Tit (Parus montanus) by means of thermocouple have indicated that these birds wh n exposed to ambient temperatures below 0°C regulate their nocturnal body temperatures as far as 10 12°C below their normal diurnal temperature (Haftorn 1972 Chaplin 1974 Grossman & West 1977) The degree of temperature lowering differs significantly but this may be due to the different techniques used for body temperature measurements The aim of the present study was to develop a method for continuous registration of core temperature of unrestrained small birds (10-12 g)

A small telemetry transmitter was developed for surgical implantation in the intraperitoneal cavity of the Willow Tit The transmitter had a transmission range of 1 m and a sensitivity of 5% per °C It was a nearly spherical disc with a diameter of 9 11 mm a height of 4 mm and it weighed about 0 60 g The signals from the transmitter were received through a commercial radio The pulse rate depended on the temperature with increasing rate for increasing temperature The transmitter was calibrated in a saline bath over the range of temperature expected in the test animal (30 45°C) The transmitter was placed in the abdominal cavity under light other anesthesi As soon as the circadian behavioral cycle of the test bird was identical to that of the control birds the experimental temperatur measurements were performed Immediately after completion of an experimental series the transmitters were removed and retested to verify calibration

The telemetric temperature r cordings were compared to simultaneous thermocouple measurements The circadian temperature cycle was correlated to the circadian behavioral cycle The time and d ration of deep sleep including the dynamics of thermoregulation when going to and waking from deep sleep were recorded

The developed transmitters provided for remote recording of body temperature of unrestrained Willow Tits The body temperature showed diurnal fluctuations and also transient fluctuations resulting from minor disturbance Hence continuous temperatur measurements are necessary if maximum degree of hypothermia shall be revealed

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D 28

Behaviour patterns i cod released by electrical stimulation f the lfactory tract bundlets

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The olfactory tract in cyprinids gadoids and silurids is long and cont ins groups of myelinated and unmyelinated fibers (Doving & Gemne 1966 Doving 1967) The various portions of the tract terminate in different regions of the central nervous system indicating different functions (Finger 1975) When timulating lectrically the olfactory tracts in goldfish Grimm (1960) observed a feeding behaviour

The caudal part of the olfactory tract of cod (Gadus morhua L) can be di sected in four di tinct bundlets Three of these bundlet were cut symmetrically on both sides so that only one of the four bundlets were intact A pai of bipolar electrodes were hooked under the tracts cemented to the skull and connected to a pulse stimulato Weak electrical pulses in the order of 0 3 to 6 0 µA and of 0 3 ms duration elicited fo each a distinct and different behaviour i the free swimming cod

Stimulation of the lateral part of the lateral olfactory tract made the fish tilt the head dow to touch the bottom with the barbel and the first rays of the pelvic fins moving backwards This behaviour seems adapted to food search for benthi prey

Stimulation at low intensities of the medial part of the l teral tract induced a forward swimming Slightly higher stimulation i tensities induced rapid snapping

When the lateral part f the medial tract was stimul ted the fi h swam around with the snout above water A quivering f the fish could be observed at high timulation i tensities

Stimulation of the medial part f the medial olfactory tract made the fish lie quietly on the bottom, and change ski coloration as if froid

These observations indicate the repertoire f behaviour patterns the olfactory system can medi ate in the cod The results support the notion of a spati l basi for lfactory discrimination and open new experimental means of investigating the neuroanatomical substrate for fish behaviour The above observations will implify the search for odorants that li it thes behaviour patterns

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The importance of low molecula weigh fac or for human sperm motil ty

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Human seminal plasma normally has sperm motility promoting ability due factors secreted by the human prostate (Lindholmer 1974a) Albumin which is secreted by the prosta could be one factor f major importance in this respect (Lindh lme & Eliasson 1974) in part due to it bility to protect the spermatozoa from "toxic ffects exert d by semi l plasma zinc (Lindholmer 1974b)

The im of the present tudy was to further characterise seminal plasma factors promoting sperm motility and survival

Seminal plasma from semen sampl (N=5) wi h were the 80% living and motil spermatozoa and with progressive motility score (PMS) f 3-4 (i. good-excellen) were prepared by centrifugation, whereafter each supernatant were divided in two part One part served as control seminal plasma whereas th othe pa was dialyzed for 48h (4 C) agains buffered sal solution, BSS (volume rati 1 1000) To BSS was changed after 12h The control wa stored for the same ime period (4 C) Thereafter one al quot of washed spermatozoa obtained from pooled semen samples (qual ty above) were incubat d for three hours (37) i each control and in ach di lyzed seminal plasma specimen Fructose and an ibiotics wa dded to ll sperm suspension Motility vi bili y and PMS were ingle blind asse sed 0 1 2 nd 3h

Th sperm liquot incubat d in th dialyzed seminal plasma had ignificantly lowe percentag motil cell than those incubated in control semina l plasma (At 1h p< 0 05 3h p< 0 01) The spermatozoa incubat d in control seminal plasma tained progressive motility wh rea those exposed dialyzed seminal plasma otally l st this quality in four out of five case) Th sperm survival % living sperma ozoa did not diff between the two groups

Thus low molecula weight componen (MW 10 000) are of ignifican importance for th sperm motility p omo ing bility f seminal plasma Furth rmore the dec eased motility of th sperma ozoa incuba d in dialyzed seminal plasma is unlikely due any oxic ffects exr rted by free inc ion re removed by dialysis Moreover prosta l albumine lone has no major rol for th mo ility promoting ability of human seminal plasma ince lbumine is retained within th dialyzing sack

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Innervation and gl tamergi o trol f neurons in l t ral ptum and nucleus f diagonal b nd i th limbi system

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The eptal complex is maj targ t ar for fibr arisi g in the hippocampal f rmation (Swanson and Cowan 1977) Re cent evidenc gg ts that on f thes fibres may us glutamat as neur transmit ter Thus gl tame gi fib may co trol neurons in both eptum and ucleus f di gonal band. (Fonnum and W laas 1978 Walthe-Sø ens en t) 1979) It was f consid bl t t t t identify the natu f th ecipient ll Thi may b achi ed by means f local injection f th eurot xi gl tamat ana-l g kaini d ich eems to de troy l t r y ne ronal ll bodi c i ing glutama gi input

Lat l inj ti f kaini id (2 ug) in l teral eptum and di go l band wa compani d by decr f 60% in th acti ity f gl tamat decarboxyl (GAD) in septum and dec s f 50% in th acti ity f choline ac tyltransf ras (ChAT) in di gonal b d No chang s could be ob rved i th high ffinity uptak f gl tamat Loc l inj ction in medi l eptum did not ff t the pr ent neur transmitter ma k in l teral eptum hippocampus How 90% decr as in the ChAT ctivity in hippocampus ould be detect d aft injection f kaini cid in di gonal band only, whe as the GAD a tivity was unchanged Simultaneously 20% decr ase in ChAT was bserved in eptum ft this injection
Bil teral fimb ia/f rnix l ion l ompa ied by 70% eduction in th high f finity uptak f glutamat i septum and 50% eduction in di gonal band This l ion protected GAD in septum g inst th neuro t xi ff t f kaini ac d In additi glutamat (10^{-3} M) was abl to lea [^{3}H] -GABA in vitro lic of l ter l ptum
Sin the GABA gic neur ns we p f anti lly d troyed in th l ter l septum by kaini id whilst protected by fim b a/f rnix l ion, th ll which rec ive gl tame gi fferents from hippocampus e mo t lik ly GABA gi eurons This i upported by the rel as f GABA by gl t mate in eptum By inf renc , th cholin-gi eurons in diagonal band i ontrol led by gl tame gi ff rents from hippo campus providing th hippocampus with a very inte ting f d back control f ch lin rgic fib e p jecti g from di go-nal band t hippocampus

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D 31

Development of neurotransmitter parameters in lateral geniculate body visual cortex and colliculus superior of rats

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There is increasing evidence for glutamate as the transmitter in the corticofugal projections from visual cortex (VC) to lateral geniculate body (LGB) and colliculus superior (CS) (Lund Karlsen & Fonnum 1978)
It is well established that the high affinity (HA) uptake of glutamate in synaptosomes is a marker for glutaminergic nerve terminals (Fonnum et al 1979) We have therefore studied the postnatal development of these cortical projections in normal rats and in rats with unilateral visual cortex ablation at birth HA GABA uptake and GAD activity were used as markers for GABAergic fibers and ChAT activity as a cholinergic marker

In LGB and VC the HA glutamate uptake showed increasing activity from birth to adulthood In contrast in SC the maximal uptake was higher at birth and reached a peak after 12 days of age ChAT and GAD activities increased with age in all 3 regions In all regions GABA uptake showed a 2 3 fold higher activity at 12 days of age than in adult animals
In some neonatal rats visual cortex was removed on one side After 2 months the HA glutamate-uptake was similar contra and ipsilateral to the lesion in both SC and LGB The uptake activity however was about 50% of unoperated adults
Such results may be explained from an aberrant crossing projection from the intact visual cortex (Mustari & Lund 1976) The ipsilateral decrease may reflect the loss of fibers crossing
These results describes the development of unilateral descendent glutaminergic fibers from visual cortex and that these after unilateral visual cortex ablation in the neonatal rat may also make contact on the contralateral side

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D 32

HDL cholesterol physical activity and diet in middle-aged men

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Previous studies have demonstrated an inverse correlation between high density lipoprotein (HDL) cholesterol and coronary artery disease (CAD)risk Cross sectional studies have shown a higher HDL cholesterol concentration in physically active populations than in sedentary aged-matched controls the latter not as lean as the former
In a preceding study (Kiens et al 1979) increased HDL-cholesterol concentrations occured in healthy non-obese initially sedentary middle-aged men after 12 weeks of physical conditioning These findings occured without changes in body weight or degree of adiposity Dietary history data showed an increase in caloric intake without changes the percentage distribution of fat carbohydrate and protein thus the absolute fat intake increased
The aim with the present study was to investigate whether the observed increase in HDL-cholesterol was primarly due to the effect of physical activity or to the increased dietary fat intake
23 healthy regularly physically active (VO_2 max 53 5 + 3 9 ml/kg x min 1 mean SD) middle-aged men were studied 10 assigned to the experimental group and 13 to a control group The experimental group were studied before after 4 weeks of fat rich and after 4 weeks of carbohydrate-rich diet
Preliminary results show no changes in HDL-cholesterol concentrations neither after fat rich diet compared to the normal before values(1 65 + o 12 vs 1 63 o o9 mmol/l N S mean + SE) nor after carbohydrate rich diet compared to normal (1 59 o 13 vs 1 63 o o9 mmol/l N S mean SE)
No changes were seen in the controls Training habits or body weight did not change during the study
This suggests that physical activity per se is the primary facto for the increase in HDL-cholesterol concentration

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Theophylline induced relea of enin f om the rabbit kidney and its inhibition by adenosine

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It is well stablished tha theophyllin incre se plasma renin activity in mammal (Win t al. 1969 and othe). One mechanism I commonly assumed to be inhibition of AMP phosphodi t ase. In this communication we repo that the phylline in concentrations that have little or no effect on renal -AMP phosphodi terase incre ses renin release and that thi ff ct is eff ctive ly antagonized by adenosine

Rabbit kidneys were isolated and perfused t constant rate with Tyrode elution (Hedqvi t et al. 1978). Renin release wa determined g angiotensin I formed pe hour and ml perf te using comme cially availabl [125]I-angiotensin I radioimmuno-assay kit.

Theophylline caused dose-dependent inc as in renin release from th kidn y A ignifi an effect was obtained with th ophylline 1μM and with 30 μM renin leas w mo than doubled. In these concentration th ophylline has virtually no effect on -AMP ph sphodie te activity in the rabbit kidney (H dqvist et al. 1978) Aden in inhibited spontaneous release of renin, and oun racted the enhancing ff of theophyllin Th inhibitory effect of adenosine on enin rel se by theophylline dose-dependent reve ibl and surmounted by rai ing th theophylline con tr tion, suggesting competitive antagonism.

It was shown (F edholm & Hedqvist 1978) that enal rve timulation can ed ade osine release which correlated well to th induced va oconstri tion. In the prese tudy enal nerve timulation sed an increased releas of renin which ould be blocked by 1 μM adenosin R nin releas wa als depre sed when th vasocon tricto re ponse to nerve timulation was enhanced by redu ing N once tration in th perfusion medium by 10%. On the oth hand rve indu d releas of renin wa nhanced afte -adrenocepto blockade which an nul the vasocon tricto response and the relea of de osine (Fredholm & Hedqvist 1978)

Ou alts indicate that adenosine inhibits pon taneou l ase of renin as well as that induced by theophylline or nerve timulation. Since deno ine xerts thi ff t in concentr tion which l of the am rde of magnitude that found to be l ased from the kidney it i po ibl that th nhancing ff ct of th ophyllin on renin rele se afl cts at least in part, an inte action with endo genous adenosin The results are als con te t with the opinion that ad nosine may play role modul tor of nin release

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D 34

Protein components of small noradrenergic vesicles and chromaffin granul s from ra

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Of the six known prot in components of th bovine chromaffin granul membrane (Winkler 1976) three hav been identified in large dens ored esicl from bovine nerv trunk; dopamine-β-hydroxylase (DBH) chromomembrin B and cytochrome b561 (Lagercrantz 1976) In th solubl contents DBH and chromogranin A have been found

Th im f this tudy was to compare th protein patterns of small dens cored esi les not previously known ith thos of chromaffin granules of th rat

Rat adrenal chromaffin granul were isolated according to Smith & Winkler (1967) and small dense cored esicles ac rding to Fried et al. (1978) The subcellular fractions were sub- jected to SDS-polyacrylamid gel lectrophore- is in 5 6% gel (W ber & Osborn 1969) Th mobility of th band relati to th tracking dye was alculated and th apparent molecular weight was d termined using standard pl t with proteins of known molecular weight

The rat chromaffin granul embran contained as major protein with imilar pparent molecular weight to bovin DBH, with dimer t 145 000 dalton and monomer t 87 000 dalton. Two ther major embrane prot ins were found t 1 0,000 and 56 000 dalton. A band t 26 000 dalton indicated possibl presence f chromo- membrin B. The solubl ontents contained two maj proteins, t 100 000 and 89 000 dalton, in ontrast to bovin chromaffin granules where there is on major prot in chromogranin A t 80 000 dalton

Th small dens cored esicl fraction also contained protein with similar mobility to bovin DBH but this was no major prot in. A trikingly large omponent was found t 28 500 dalton, the mobility of chromomembrin B N major prot ins with mobilities imilar to hromogranins were pre ent.

Th small noradren rgi esicles hav previous- ly by biochemical method been shown t contain DBH and ytochrome b561 (Fried t al. 1978, Fried 1978) These results indi te that also chromomembrin B is present that DBH is not major prot in and that chromogranins are very mino components if pre ent t all. Regarding chromogranins there eems to be differenc b tween rat and bovine chromaffin granules

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D 35

Respiratory functions in highly trained - and normal boys during puberty

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The aim of the present study was to evaluate the qualitative changes in the following respiratory functions Vital capacity (VC) maximal pulmonary ventilation (V_E) maximal tidal volume (V_T) and maximal respiratory frequency (f)
These parameters were measured in a group of six highly trained boys (group 1) and in a group of 5 normal boys (group 2) from 1 5 years before till 1 5 years after peak height growth velocity (PHV) (for further details see Andersen and Froberg 1979)
VC measured in supine position was found to be 10% lower in group one than in group two before PHV From PHV occurred until 1 5 years after the two groups were similar Corrected for diff rences in height ($VC=a \times h^3$) similarity was found until one year before PHV Then VC increased relatively more in group one resulting in a 10% higher VC in this group from 0 5 years after PHV
Maximal V_E in group one increased linearly from 85 1 x min^{-1} 1 5 years before PHV to 135 1 x min^{-1} 1 5 years after The corresponding values in group two were 75 1 min^{-1} and 115 1 x min^{-1} This increase was not linear The difference in V_E between the two groups was not related to differences in maximal V_T but alone to differences in f The later was 66 5 in group one 1 5 years before PHV and decreasing to 60 3 1 5 years after PHV In group two they were 55 7 and 49 5 respectively The ventilatory equivalent was close to 38 liter per liter oxygen in both groups during the period
The results indicate that training during puberty induces qualitative changes in VC and in f This could partly explain the higher maximal oxygen uptake found in the highly trained group

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D 36

Circulatory parameters and muscular strength in trained and normal boys during puberty

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The present paper presents circulatory parameters and muscular strength measurements in a group consisting of six highly trained boys (group one) training more than 7 5 hours pe week and in a group consisting of 5 normal boys (group two) training less than 3 5 hours per week over a period of 6 years starting at the age of eleven years The obtained measurements were related to the time of peak he ght growth velocity (PHV) Comparisons between the two groups are made from 1 5 years before PHV till 1 5 years after (For further details on VO_2 and respiratory functions see Andersen and Froberg)
The hematocrit (Hct) values was lose to 40% during the whole period and was the same in the two groups while hemoglobin concentration (Hb) increased from 13 to 14 9 gram per 100 ml in the same period The increase was similar in the two groups except during PHV and o 5 years before Here the Hb concentration was constant in group one while it increased constantly in group two In group one the concentration was 95% of the values found in group two The average maximal HR was 198 in group one and five beats higher compared to group two If corrected for height the difference was 1 2%. Net efficiency was found to increase from 21 5% to 22 5% at the submaximal work loads over the years investigated with no systematic differences between the groups Maximal isometric strength was measured in a strain gauge apparatus The strength in the back muscles was found to increase from 460 N to 740 N in group one This was 30% higher than found in group two From 1 5 year before PHV to 0 5 years after PHV the difference between the groups was only 10% if corrected for differences in height ($F=a \times h^2$) The same pattern was found for the elbow flexors
The study indicate that when the puberal growth spurt takes place highly trained boys have less advantage of the training of muscle strength compared with the period before and after PHV This is in accordance with results obtained in a cross-sectional study (Lammert et al) The circulatory changes are in accordance with the literature except for the 5% lower Hb concentration values in group one It may reflect an adaptation of blood viscosity to training

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[illegible] oxygen uptake and lactate concentration in [illegible] trained and normal boys during puberty.

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Results obtained in literature are contradictory concerning the effect of training on boys during puberty (Eblon 1979 Kobayashi et al 1978 Weber Kartodihardjo & Klissouras 1976) One reason could be that results generally are related to chronological age not to development. The present longitudinal study deals with 11 boys (out of group of 29) investigated over period of 5 years starting at the age of 11 years. Six of the boys (group 1) trained more than 7 5 hours per week. Most of the training partly supervised by this institute was performed in soccer club. These boys were compared with five boys (group 2) who trained less than 3 5 hours per week.

Height, weight oxygen uptake pulmonary ventilation, heart rate and blood lactate concentration were measured every half year.

The tests were performed on bicycl ergometer at two submaximal and t supramaximal work loads. The changes in maximal oxygen uptake and lactate concentration were related to that half of year where the increase in height was the highest (Peak Height growth Velocity PHV) It was possible to compare the results from all the boys in the two groups from 1 5 years before PHV until 1 5 years after

It was found that maximal oxygen uptake in group one increased from 2 2 1 O_2 min^{-1} to 3 5 1 O_2 min^{-1} in the period constantly being 250 ml O_2 min^{-1} higher than the values obtained in group two Concerning blood lactate concentration, the maximal values were found to be 10 mMol 1^{-1} in group one over the period In group two the values were 7 5 mMol 1^{-1} 1 5 years before PHV increasing till 9 mMol x 1^{-1} 5 years before PHV and being so the rest of the period.

If sensitivity to training is defined as change in the trained parameter per unit of time the two groups were equally sensitive concerning maximal oxygen uptake regardless of the greater amount of training performed by group one In regard to maximal blood lactate concentration, this was only so from 5 years before PHV

This suggests that an external stimulus such as training apparently can not override the influence of growth during thi period. This may be discouraging but could be outweighted by the qualitative changes described in the f llowing papers

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The relationship between isometri strength in [illegible] and girls and developmental parameters.

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Muscle strength is related to several parameters [illegible] muscle fiber types, degree of training, sex and age Before puberty there is hardly no difference between the two sexes and only slight annual increase in strength.When puberty occurs boys start to increase muscle strength rapidly while thi is not seen in girls During this period chronological age is poor indicator of development. The present study investigated the relationship between maximal isometri strength and to the following parameters chronological age height weight skeletal age dental age Tanners adolescent stages (Tanner 1962) and predicted onset of puberal growth spurt These parameters were measured in randomly selected group of 234 children chronological aged 9 to 17 years. Each age group had an equal number of boys and girls. The strength in the arm flexors (lbow angle 90°) was measured in strain gauge apparatus. Skeletal age (Greulich & Pyle 1959) dental age (Demirjian et al 1973) and predicted onset of puberal growth spurt (Hx)(Helm et al 1973) were obtained from radiograph of the hand and of the teeth.The results show that for the boys maximal isometri strength increased up to 12 years of age and again t 13 5 years of chronological age A similar pattern was also found when isometric strength was related to height weight and predicted onset of puberal growth spurt Here the lack of increase in muscl strength was seen from 154 cm to 162 cm 42 kg 47 kg and 1 5 years before Hx to HX respectively Compared to skeletal age it increased up to 12 years with an increase like the one seen in the girls and then with steeper increase The girls allways had somewhat lower muscle strength than the boys The relationship for the girls showed constant increase up to 2 years after Hx when isometric strength became constant When related to chronological age weight height or skeletal age the strength increased constantly within the observed intervals.

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D 39

The relationship between isometric strength in girls and boys and the serum concentration of FSH

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Muscle strength is related to several parameters such as the degree of training age and sex Strength increases aligthly until the age where puberty occur then it is well known that muscle strength increases rapidly in boys It is also known that during this period changes occur in the serum concentration of several hormones The present study investigated the relationship between isometric muscle strength and the serum concentration of FSH (follikel stimulating hormone) Measurements of maximal isometric muscle strength in the arm flexors at 90° elbow flexion and blood samples were obtained twice interspursed by 5 weeks A randomly selected group of 91 girls and 118 boys aged 9 to 17 years were used No systematic change in strength or FSH was found between the measurements FSH was measured using a radioimmunoassay method The relationship between force and FSH can be expressed for the girls as force 0 058 x FSH concentration 9 6405 with a correlation coefficient of r=0 443 and n=182 For the boys it was force 0 3544 x FSH concentration 10 2118 and r=0 6423 and n=236 The relationship was found to be linear for both boys and girls The values of FSH for boys were between 2 2 and 20 m IU/ml and the force between 60 and 250 N For the girls the values observed were between 3 3 and 29 m IU/ml and the force between 59 N and 185 N The linear relationship found for the boys are not in accordance with results obtained when force is related to chronological age onset of puberal growth spurt or skeletal age (Lammert et al 1979) For the boys the statistically explained variation was 40% while it only was 20% for the girls In both cases the relationship was highly significant $t_{♂}$=12 88 and $t_{♀}$=6 64 and P< 0 001

In conclusion the investigated relationship reflects poorly the changes in muscle strength in this cross sectional group One of the reasons could be that increase in serum level of FSH is reported to start in the prepubertal period (Ferin et al 1974)

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D 40

Release of endogenous noradrenaline from blood perfused rabbit kidney Influence of prostaglandin E_2 and indomethacin

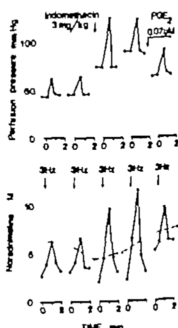
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The hypothesis that prostaglandins of the E type (PGE) play a significant modulatory role in adrenergic neuroeffector transmission (cf Hedqvist 1977 Ann Rev Pharmacol Toxicol 17 259 279) was tested in blood perfused rabbit kidneys in situ Kidneys were perfused at constant rate (10 ml/min) and the renal nerves were intermittently stimulated (3 Hz 1 msec for 30 sec) Noradrenaline (NA) in renal arterial and venous blood was determined by high performance liquid chromatography and electrochemical detection

Renal nerve stimulation caused vasoconstriction and increased NA in the venous effluent by 7 6 ± 1 1 nM (n 22) Indomethacin (3 mg/kg i v) increased renal perfusion pressure and enhanced both the stimulation-induced outflow of NA and the vasoconstrictor response (Fig) NA outflow was increased by 87 ± 30 % (n 5 p 0 05) PGE_2 (0 07 µM) reduced stimulation-induced NA outflow to 51 ± 9 % (n 5) of control values and attenuated the vasoconstrictor response The effects of PGE_2 were similar after treatment with indomethacin

The present results obtained with determination of endogenous NA in the blood perfused kidney in situ provide considerable support for the view that PGE_2 may be a significant feedback modulator of transmitter secretion in adrenergically innervated tissues



Effects of indomethacin and PGE_2 on responses to renal nerve stimulation

Supported by grants from SMRC (4342) Swedish Soc Med Sci and Karolinska Institutet

Permeability of the peritubular capillary membrane in the rat kidney

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The fluid reabsorption across the peritubular capillary membrane will be governed by the Starling forces operative across the membrane and its permeability. The permeability of the membrane was analysed from its hydraulic conductivity and its permeability to 125-I-paraaminohippuric acid (PAH) 3H-inulin, albumin and gammaglobulin

Hydraulic conductivity was investigated from the driving forces and fluid transf Hydrostatic intracapillary and interstitial pressure was measured with direct punctures. Interstitial colloid osmotic pressure was determined from the protein concentration in renal hilar lymph. Oncotic pressure along the peritubular capillary was calculated from the protein concentration in efferent arteriolar blood and in renal venous blood. The fluid transfer was determined from single nephron filtration and the inulin concentration in distal tubular fluid.

During antidiuretic conditions the mean net driving force was 14.1 mm Hg and the amount of fluid reabsorbed 13.5 ml/(min 100 g rat) i hydraulic conductivity of 0.96 ml/(min 100 g mm Hg)

This would mean total pore area over pore length Ap/dx of about 2 cm/ 00 g at as calculated from Poiseuilles law for radii between 3 and 15 nm.

From microinjections of PAH into the peritubular capillary blood the flux across the membrane could be calculated and this resulted in Ap/dx of about 0 5 cm and was also shown to be very unsensitive to the pore radius. The transport of the protein was derived from the concentration in systemic plasma and hilar lymph. Albumin and gammaglobulin concentrations was determined seperately from their concentration ratio. The permeability Ap/dx was here estimated 2 and 3 cm respectively for 10 nm pore

Combining all data the total pore area for pore length of 0.1 µm was estimated to 2 10^{-3} cm^2 corresponding to about 1 % of the total capillary area. The effective radius of the pores for reabsorption will range from 3 to 7 nm.

During extracellular volume expansion the driving forces decreased but the permeability increased in proportion resulting in an unchanged absolute reabsorption

It is concluded that the permeability of the peritubular capillary membrane is very high compared to that of other capillary beds but very similar to that for the glomerular capillary membrane

Evidence for both Na and Ca^{2+} dependent hyperpolarizing current in Helix pomatia neurones

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Many of the neurones of Helix pomatia display particular so-called impulse dependent firing pattern (Colding-Jørgensen 1975) because each firing interval depends on the presence and timing of previous impulses

This behaviour is due to accumulation of hyperpolarizing current which is triggered by the depolarization during the impulse and which decays exponentially after the impulse. The reversal potential of the current is −60 to 70 mV which strongly suggest that the current is carried by K^+ ions (Colding-Jørgensen 1977)

Further analysis has revealed that the current vanishes in Ca^{2+} free medium and that Co^{2+} reduces the current to less than 10% without changing the reversal potential. The first observation implies that the current depends on the extracellular Ca^{2+} concentration the second that influx of Ca^{2+} plays an important role in the current formation. However the Ca^{2+} does not participate in the current itself

Replacing Na^+ with $Tris^+$ results in a reduction of the current to 40-50% of its normal value but also in hyperpolarization of the reversal potential of 5-15 mV. This implies that extracellular Na^+ is necessary for the full current but also that Na^+ participates in the current itself. Replacing Cl with isethionate does not give any significant change in the current

The decline of the hyperpolarizing current after depolarization of 50 mV is in all cases mono-exponential so the *current is most probably passing through* homogeneous population of pathways

It can therefore be concluded that the current appears to be carried through the membrane by mechanism which is depending on the presence of both Na^+ and Ca^{2+} in the bathing medium and that the current mainly is carried by K^+ ions but to small extend also by Na ions. Whether the controlling ions act on the outside or the inside of the membrane is not yet determined. Preliminary experiments show that the intracellular Ca^{2+}-concentration increases during the depolarization and decreases the current decreases but the effect of Na is still unknown

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D 39

The relationship between isometric strength in girls and boys and the serum concentration of FSH

O LAMMERT K FROBERG & JØRGEN ROOS

Institute of Physical Education Odense University & Danish Seruminstitute Copenhagen Denmark

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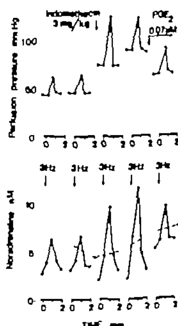
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D 41

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D 42

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Replacing Na with Tris results in reduction of the current to 40 50% of its normal value but also in hyperpolarization of the reversal potential of 5-15 mV. This implies that extracellular Na^+ is necessary for the full current but also that Na^+ participates in the current itself. Replacing Cl^- with isethionate does not give any significant change in the current

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It can therefore be concluded that the current appears to be carried through the membrane by a mechanism which is depending on the presence of both Na^+ and Ca^{2+} in the bathing medium and that the current mainly is carried by K^+ ions but to a small extend also by Na ions. Whether the controlling ions act on the outside or the inside of the membrane is not yet determined. Preliminary experiments show that the intracellular Ca^{2+}-concentration increases during the depolarization and decreases as the current decreases but the effect of Na is still unknown

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D 43

Local cerebral glucose consumption during a single cortical spreading depression

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Leão Co tic l sp ding D pr io (CSD) f EEG i a g li d r p of th r b l o t t io i flu c Th CSD i mp i d by l c l ch g of DC p t ti l and t cellul c nt ti n f K+ Na+ Cl d Ca++ d m v t p d of 3 mm/min c o th o ti l f Th po f th c b l m t b li t th p g of i gl CSD h n v p i ly b po ted lth gh vid of i c d gl s pti w obtai d in p ted li it tio of CSD f r 45 min (Shinohar & al 1979)

I d t tudy th t mp l l ti hip b tw n b l blood fl w (rCBF) gl o mpti n (rCMRG) d bl od b i b i r gl t p t p ity (Tmax) i pidly ch gi g f ti l st t th v iabl w m d i 6 m l Wi t t by i di to f cti ti (Gjedde & al 1979) A th i w b t i d with p t b bit l (50 mg/kg) Th b i w f i it d i g th p g of i gl CSD 10 ft i t v b l i j ti f lab l d b t l d D gl d l 2 mm l li w t f th t Th [K] w m i to d with i l tiv mi l t od

Tmax (and Km) w re lcu l t d by v vs v/s pl t i nim l with t ri l pla a glu s c ntr tio s f m 2 to 32 mM rCMRG w c lcul t d f Tmax Km a d co t ti s f gl os i pl nd b in Th r g v l e ob t i d i th di t r b d ight h i ph giv i th t bl (±SD)

rCBF	44±2	ml/100g/min
Tmax	157±11	µmol/100g/min
rCMRG	29±5	µmol/100g/min

Th ch ng ec- ing i th di t b d l ft h i ph i p r t f th ight h i ph h w i th fig i hich p ti l v ts w t d t t p l v t by th k ow t f p gr i f th CSD-w

Th fig how th t th i iti l t i 3 f ld i f rCMRG f ll d by 2 f ld i of Tmax nd rCBF M i l h g of rCMRG d rCBF ob d du- ing th n m li ti f [K]

Th lt how th t th bl od b i b i t p t p ity i m kedly d ing i d t b li d th t h g f flow d tab li y b di ci t d i ti

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D 44

The basem nt membrane thickne and mitochondrial conte t of brai capillaries in normal d po tocav l hu ted rats

Laursen H

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The volume of mitochondria in brain capil lary endothelial cells exceeds the content in capillary endothelial cells from a number of other rat tissues and an excees metabolic work capability of the blood brain barrier has been ugge ted (Old dorf et al) In order to define the structural relationship of the blood brain barrier to work capacity and diff sing capacity in hepatic encephalopathy where changes in the barrier may be an etiologic factor the mitochondrial content of the endothelial cells and the harmonic mean thickness of the basement memb ane of the brain capillaries were mea urod in sham operated and portocaval shunted rats

The mitochondrial content wa measured by automatic image analysis (Laursen & Diemer) and the harmonic mean thickno of the brain capillary basement membrano was determined from orthogonal intercepts measured on a reciprocal logarithmic sc le as proposed by Jensen et al The mitochondrial content in brain capillary endothelial cells was 5 97 (3 25 8 75) per cent after sham operation 7 64 (3.57 lo 7o) per cent after lo days PCA 5 78 (3 55-8 51) per cent after 3o days PCA and 5 73 (2 6o-lo ol) per cent after lo we ks PCA (median with 25 75 percentiles) The harmonic mean thicknes of the ca pill ry basement membrane wa 57 8 nm after sham operation 54 6 nm after lo days PCA 63 2 nm after 3o days PCA and 6o o nm after lo weeks PCA The slightly incre ed val e of the basement memb ane thickness after 3o days PCA was the only diff rence betweo all the groups

It is concluded that only a slight change in diffusion c pacity is seen fter 3o days PCA d no other change are se i metaboli work and diffusion capa citie on the st ctur l level i portosy temic encephalopathy

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Subgrouping of skel t l muscl fibres in man

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Mammalian skel tal muscl may be separated into two major fibre type based on 1) contractile properties 2) hi to chemically demonstr ted myosi ATP- se activity These two fibr type have bee classified as low twitch r Type I and fast twitch Type II respectively Recently two distinct subgroups within the Type II fibres have been demon tr ted (Brooke & Kaiser 1970) Howeve it ha proved to be difficult to cla ify 11 fibres into these three groups The p pose of this tudy we to las ify 11 fibres

We have investig ted the lability f the myosin ATP-ase enzymes in human sk le tal muscle using the f llowing proced re 1) Preincubation at arious pH between 9 9 and 10 5 and cal ium concent tions between 0 150 mM f llowed by 2) lde hyde fixation (5 mi in 2 % f rmaldehyd at 5° C) and 3) second preincubation t various pH between 4 0 to 5 0 and cal ium concentration f 20 mM 4) Myosin ATP as activity was demonstrated using the proce dure of Padykula & Herman 1955 (routine incubation)

A impl photomet i method was deve loped to meas re the optical density of the hi tochemical t i i each fibre

Using the f llowing procedure 1) Pre incubation t H 10 3 and c l ium concen t tion t 75 mM followed by 2) fixation 3) preincubation t H 4 6 and 4) routin incubation the Type I fibre were sepa ted i to three groups

After th f llowing proced 1) P e incub tion t pH 10 1 nd cal ium concen tr tion of 40 mM f llowed by 2) fixation 3) preincubation t pH 4 20 and 4) routine incubation the Type II fibre we epara ted i t three groups

On the ba i f these esults it i sugge ted that human k l t l mus l fibre hould be l ified Type I A I AB and I B and Type II A II AB nd II B

Ref rence

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D 46

A compari at rat b o ss y for l it ni (Cl) using measureme t of ioniz d al ium (Ca^{++}) in serum

L.AUKE

Department f Phy l gy and Biochemi try Dental Faculty Un versity f Osl Norw y

The polypeptide hormon CT ha l ium lowering ffect in animal Th phy i l g cal ignifica ce f CT i not l ly un derstood and ff rt this gard have been hampered by lack f it bl methods for asse ing t bi l gi l ctivity

Previously publ hed methods ll based on the b lity f CT t produce hy pocalcaemi and they in l d ff rent dietary gimen,diff rent pe and g of the as ay animal well iant routes f admini tration and time int vall fo blood coll t and det rmina tion f tot l rum cal um

The present ay procedure involves special d t ry regimen and CT lnt on or test ampl j t d ubcutan ous ly The prin p l diff re between thi and othe b as y i th determi t on f serum ionized l um nst ad f tot l cal ium

Ion ed cal ium & me ured by Cal i um Ion Sel trode pe i l rrang ment developed f serum analy The Ca Selectrode onnected t pH-met i serie ith micro pH-elect od Bef re serum ampl measurement re made cali bration curve f the Sel trode must be pl tt d Corresponding value f r me ured pot ti l (mV) re pl tt d on semil g thm paper g t th cal ium ion on t t f the standa d lution used Th rum ampl re handl d an obic lly (V cut ine) and th pH and lc um ion concentration r re d i th ame ample The hypoc lcaemi re po t CT in young Wi t rat (100g) were ompa d f both ioni d and t t l rum cal ium me rement

Do f % r duction f rum c lcium CT mean S E ( ) 1h ft c g i jection f CT (Cibacalcin)

	T t l Ca	Ioni d Ca
0 05	8 5 2 5 (8)	20 0 5 0 (8)
0 10	8 5 1 0 (9)	27 5 4 0(12)
0 50	14 0 2 0 (5)	35 5 2 0(12)
1 00	16 0 1 0 (3)	39 5 5 0 (4)

gn f can $p < 0\ 05$ ff en f on ont l

The reduction f serum ioni d Ca i 2 3 t me gre t than the chang in th t t l rum cal ium

Th bioassay h ch i conve ient rapid and mo itive than p viously de d re b d methods has been used f t ting th bi l g cal tivity f immun tive CT i and tumour xtract from p ti ent and rats ith CT p oducing tumours

D 47

Control of muscle properties by pattern of impulse activity

by

T Lømo* R H Westgaard+ and L Engebretsen*

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Cross reinnervation experiments have establish ed that neural influences largely determine the differentiation of mammalian skeletal muscle into fast and slow types (Buller Eccles & Eccles 1960). However experiments with innervated muscles do not distinguish between transformations in the muscle caused by changes in muscle activity per se or by changes in flow of trophic substances resulting from altered nerve activity Earlier experiments on denervated muscle have shown that transformations occur with different patterns of stimulation in the absence of neural influences (Lømo Westgaard & Dahl 1974) In the present experiments this result is confirmed and extended by showing that denervated muscle may obtain very different contraction speed strength and endurance dependent on the particular pattern of activity imposed

Stimulating electrodes from external stimulators (platinum plates or teflon coated steel wire) were implanted around the denervated soleus muscle of male Wistar rats Stimulation was maintained for 3 to 15 weeks In the acute experiment measurements were made at optimal length and at a temperature of 35 36°C The soleus was either freed extensively (Lømo & al 1974) or subjected to minimal dissection of the distal end to preserve blood supply and obtain a reliable measure of endurance

Four patterns of stimulation were used, namely brief 100 Hz trains (0 67 sec) repeated either frequently (every 67 sec) or infrequently (every 1 h 67 min 50 sec) giving mean frequencies of 1 and 0 01 Hz; and 10 Hz trains (9 sec every 10 sec or 1 sec every 70 sec) mean frequency 9 and 1 Hz Muscles stimulated with trains of 100 Hz became fast while those stimulated with trains of 10 Hz remained slow irrespective of mean frequency The *change in contraction speed resulted in the steep* part of the tension vs frequency curve (where muscle tension is most sensitive to small changes in rate of stimulation) moving towards the imposed frequency Muscles receiving frequent stimulation *became stronger and more fatigue resistant than* those stimulated less at the same frequency Muscles stimulated at 100 Hz produced larger tetanic force than muscles receiving much more frequent stimulation at 10 Hz

These results show that the muscle is able to adjust a number of intrinsic properties according to the external demand on it Frequency and amount of stimulation are important factors in determining the new intrinsic muscle properties

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D 48

The role of adr naline f panc ti hormonal secretion and muscular glycogenolysi i running t

B SONNE E A RICHTER & H GALBO

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During prolonged rcise insuli secr tion decrease and glucagon secretion increases We have eported that i swimming ts the hormonal change ar diminished fte adrenodemedull tion (DM) Compared to contr l DM- ts also have highe muscular glycogen conc t re t and dimini hed glycogenolysi during swimming (Richte t l 1979) These ffects f DM in swimming ts might be secondary t changed swimming intensity ffi iency

We have now tudied if imil ffects f DM an be demonstr ted in running and if they an be eversed by exogenous adrenaline 24 ts we DM and 16 sham-oper ted (C) Arte i l and venous theters were implanted 2 wks lat the ts ithe ested (16) for 45 mi or an i metabolism chamber (24) (Sonne t l) 15 min t 10 mi ^{1} nd 30 mi t 14 min ^{1} (grade 0%) Running DM- t had saline (DM S) or adrenaline (DM A) (to the same pl ma conc as in C- ts) infused Blood sampl were drawn t re t and during exer ise Immedi t ly f ter the 45 th mi soleus muscl samples we tak

V_{O_2} was identical i C and DM- t incr asing from 2 4 ± 0 1 (mean ± S E) t re t to 4 9 0 1 ml O_2 (STPD) (min 100 g) ^{1} during exerci I C ts heart te (HR) incre sed from 394 ± 14 t 469 ± 13 beats mi ^{1} during ex rcise In running DM-ts HR only incre sed ignificantly i DM A ts (from 366 ± 13 t 445 ± 15) I C and DM-A ts blood glucose increased from 6 0 ± 0 1 mM to 8 2 ± 0 5 and 7 0 ± 0 3 respectively whe eas it decreased to 5 6 ± 0 2 i DM-S t during exercise Plasma insuli conc was the same i re ting and running r ts In C ts the pl ma glucagon conc increased from 11 2 t 34 6 pM during exe ise and in DM-A and DM-S ts from 13 ± 2 to 29 3 and 28 2 respectively Ev luating these hormonal changes rel tive to th changes i blood glucose it appears that compared t DM-S r ts insuli conc was depr sed and glucagon conc increased i C and DM-A ts during running I C ts muscular glycogen decre sed from 17 ± 1 to 9 ± 2 mmol kg ^{1} during ex ise and i DM-S and DM-A r ts from 27 2 t 19 2 and 10 1 respectively the de cre being large i DM-A than i DM-S ts I C ts blood lactate increased from 1 36 0 10 t 2 37 0 46 mM du ing ex rcise and i DM-S and DM A t from 1 14 0 05 t 1 51 0 09 and 2 33 ± 0 15 spectively the incr se being large i DM-A than i DM-S ts

I conclusion the ffect f DM on the changes i pancreati hormonal sec tion and muscular glycogenolysi du ing exe ise i not secondary to changes i ove ll metaboli t During ise adrenaline ss ts an acut nhancing ffect on muscula glycogenolysi glucagon se ion and an acute depr ssing ffect on ins li sec etion

R f ences

RICHTER E A GALBO H SONNE B HOLST J J CHRISTENSEN N J 1979 Ac Phy i l S and i p

SONNE B & GALBO H Act Physi l Scand Suppl thi volume

Simultaneous determination of oxygen uptake heart rate and rectal temperature in running rats

SONNE GALBO

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The rat is extensively used as an experimental animal in exercise physiology Nevertheless there is shortage of information about the relationship between work intensity and basic physiological parameters obtained steady state in exercising rats.

We have now developed metabolism chamber including tiny treadmill for determination of oxygen uptake ($\dot{V}_{O_2}$) heart rate (HR) respiratory exchange ratio (R) and rectal temperature (T) in running rats. Furthermore arterial blood sampling and intravenous infusion can be made through chronically implanted catheters

In resting rats $\dot{V}_{O_2}$ and HR were 2 3 0 2 ml O_2 (STPD) (min·100 g)$^{-1}$ (mean S.E. 8) and 394 14 beats·min^{-1} respectively

Untrained rats ran at an inclination of 0% and running speed of 10 m·min^{-1} for 31 min. During the initial 19 min $\dot{V}_{O_2}$ HR and R decreased from 4 9 0.3 ml O_2 (STPD) (min·100 g)$^{-1}$ (n 8) 452 21 beats·min^{-1} and 0 89 0.03 in the 7th min to 4.3 0 2 421 17 and 0 83 0 02 respectively in the 19th min (0 05) whereas T_R remained unchanged 38 8 0 2 °C This initial overshoot" did not diminish during the four times the rats within period of 10 days participated in exercise experiments None of the measured parameters changed from the 19th to the 31th min. After 11 min of exercise the inclination was set t 0 5 10 or 15 and the speed was stepwise increased with 5 m·min^{-1} every 11 mi At all inclinations $\dot{V}_{O_2}$ HR R and T increased significantly with increasing running speed No levelling off of $\dot{V}_{O_2}$ and HR was observed. The highest measured $\dot{V}_{O_2}$ HR R and T_R were 8 6 0 3 ml O_2 (STPD) (min 100 g)$^{-1}$ 556 16 beats·min^{-1} 0 92 0 02 and 40 0 0 4 °C respectively No differences in $\dot{V}_{O_2}$, HR, and T were found between experiments diff rent inclinations and identical speeds

The present tudy has shown tha in order to carry out work physiological investigations in rats it i necessary to introduce 20 min pre-test period during which unspecifi tress responses to handling and new environment disappear Thi pre-test period can not be replaced by "habituation runs on 3 preceding days Furthermore using rats running speed rather than inclination ha to be changed in order to establish physiologically signif cant differences in work intensity Taking these findings i to account the developed metabolism chamber makes i possible to carry out sophisticated studies f metaboli hormonal and cardiovascular adaptations to exercise in rats and to relate the responses to oxygen uptak (Sonne et al)

Ref ence

SONNE B RICHTER E.A. GALBO H Acta Physi l Scand Suppl thi volume

Formation and inactivation of adenosine in fat cells Effect of propylthiouracil (PTU) treatment.

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Fat cells from hypothyroid animals are less responsive to lipolytic stimuli than cells from euthyroid animals. The mechanism behind this defect is not settled It does not involve decrease in the number of β-adrenoceptors or adenylate cyclase not an increase in phosphodiesterase but may involve defect in the coupling between the β-receptor and the cyclase (Malbon et al 1978) Recently, Chiasalo & Stouffer (1979) showed that adenosine deaminase (ADA) which inactivates adenosine that does cause an uncoupling between the receptor and the cyclase (see Fredholm 1978) restored the lipolytic sensitivity to adrenaline The adenosine antagonist theophylline has imilar effect (Malbon et al 1978) Using isolated rat fat cells we have therefore examined the possibility that drug-induced hypothyroidism (PTU in the drinking water for three weeks) is associated with change in the formation or metabolism of adenosine

PTU-treatment shifted the dose-response curve to noradrenaline (NA) about one order of magnitude to the right. In agreement with the above mentioned results ADA (1 mg/ml) did restore the lipolytic sensitivity to normal in the fat cells from treated rats However, in the control fat cells ADA caused shift of the dose effect curve to the left so that the difference between control and PTU-cells remained even in the presence of ADA.

The rate of accumulation of adenosine+inosine was higher in the cells from PTU-treated rats than in control cells under basal conditions (115±20 vs 48±8 pMol/30 min/10^6 cells) In the presence of NA (1 µM) the accumulation was higher in both groups (140±13 vs 82±15)

Adenosine is metabolised ither by deamination to inosine and further or by uptake into cells and phosphorylation to AMP ADP and ATP The latter process had K_m of 1 2 2 µM and V_{max} of 5-8 pMol/min/10^6 cells There were no significant differences between control and PTU-cells The deaminating activity of the bovine serum albumin used in the incubation was much higher than that of the fat cells Under ordinary incubation conditions (100 000 cells/ml 3% albumin) the activity contributed by the albumin was about 15 times higher than that present in the fat cells

The present results indicate that drug-induced hypothyroidism is associated with an increased rate of adenosine formation, with littl or no change in adenosine metabolism. An increased influence of adenosine may contribute to the well-known β-adrenoceptor subsensitivity in hypothyroidism especially if the sensitivity of cells to adenosine is increased (Chiasl & Stouffer 1979)

Supported by Swedish Medical Research Council (2553) Magnus Bergvalls foundation and Karolinska Institutet.

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D 51

Sympathetic regulation of adenosine in the rabbit heart

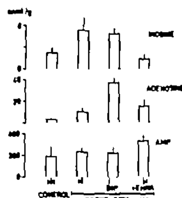
BERTIL B FREDHOLM LOUISE VERNET PER HEDQVIST* and MARIANNE WENNMALM*

Departments of Pharmacology and Physiology* Karolinska Institutet S 104 01 Stockholm Sweden

Adenosine is considered a physiologically important coronary vasodilator (Berne & Rubio 1974) Adenosine may also act as an inhibitor of the inotropic and metabolic effects of catecholamines (Schrader et al 1977) and of the release of catecholamines from cardiac nerve endings (Hedqvist & Fredholm 1979) We report the frequency-dependent increase in adenosine levels and overflow by sympathetic nerve stimulation in rabbit hearts

Rabbit hearts were perfused according to Langendorf with oxygenated Tyrodes solution and their sympathetic nerve supply were stimulated (Hedqvist & Fredholm 1979) The perfusates were continuously *collected and analyzed for purine nucleoside con*tent by reversed phase high performance liquid chromatography In addition hearts were freeze clamped and contents of adenosine inosine guanosine and AMP assayed

The effect of sympathetic nerve stimulation (10 Hz 2 min) on cardiac levels of adenosine inosine and AMP are shown in the Fig A 3-fold increase in cardiac adenosine with no significant alteration in AMP is shown In the presence of dipyridamol a further 3 fold and in the presence of an adenosine deaminase inhibitor (EHNA) a 1 5-fold increase in adenosine content is seen Overflow of adenosine and inosine into perfusates was increased by nerve stimulation The increase was related to frequency



(1 25-10 Hz) and length of stimulation (0 5-2 min) Overflow of adenosine was markedly enhanced by dipyridamol and EHNA. The amounts of adenosine appearing *in the perfusates are similar to those required to* produce significant inhibition of noradrenaline release and of cardiac catecholamine actions supporting a feedback regulatory role of adenosine

These studies were supported by Swedish Medical Council Research (2553 4342) Karolinska Institutet & Magnus Bergvallis foundation

BERNE R M. & R RUBIO 1974 Adv Cardiol 12 303 317

HEDQVIST P & B B FREDHOLM 1979 Acta physiol scand 105 120-122

SCHRADER J G BAUMANN & E GERLACH 1977 Pflüger s Arch 372 29 35

D 52

Similariti s betwe ynapti action f Ib tendo rgan ff t and I mu l pindl qff te upo pi al mot neuron

By E JANKOWSKA R HACKEL & D McCREA

Department f Physiol gy Univer ity of Göt borg Göt borg Sweden

Group I inhibition f homonymous and syn r gistic motoneurones and group I oligosynaptic excit tio have been att ibuted to the action of Ib tendon organ aff rents Recently it has been shown (F tz et al 1979) that group I muscl spindle ff re ts con lso cont ibut to the autogenetic inhibition of homonymous motoneurone The pres nt res lts tend this obs rvatio and show that I ff r nt contri but both to the i hibition f synergist and to the oligosynaptic excitation of motoneurones as well

Sinc small (< 40 µm) brief triangular stretches of triceps a ree el ctively acti ate the Ia afferents (Brown et al 1967) this technique was used t evok synaptic ac tions of Ia fferents i the motoneuron s which innervate the following mus l s of the cat hindlimb; post bi eps-semitendino gracilis, semimembranosus ant biceps quad i ceps tibial flexor digitoru longus, and peroneus t rtius and brevis Under chloral anaesthesia and muscl paralysis the tricep urae w s tonically stretched to an i itial tension of 5 N, the bri f stretches applied and intracellular effects recorded from lumbar moton urones A signal averager aided in the d tection of small amplitude postsynaptic f-fect The r s lts d monstrat th t a variety of oligo ynaptic ffects result from activation of o ly the Ia muscle spindle affere ts The segmental latencies of thes ffects (1 1-4 ms) a e compatible ith di and trisynaptic link ages i these pathways (Eccles et al 1957) IPSP wer evoked in large proportion of the e ten r motoneuro hil EPSPs predominated in the flexor motoneurones Thi p ttern is si ilar to the on described for the actions of Ib tendon organ ffer t (Eccles t al , 1957). A number f motoneuron we e found which dis played both oligo ynaptic IPSP and EPSPs) a r sponse not des ribed for actions of the Ib affer ts The e r ults together ith previous bs rvati s on the conv rgenc of group Ia and Ib eff e t upon certai int rneurones (Jankowsk t al , 1978) gge t that common int r neurones may be involved i mediating the ac tion of I and Ib ff ts t motoneurones

R f ren s

BROWN, M C ENGBERG I & MATTHEWS P B C 1967 J Physiol 192 773-800
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FETZ E JANKOWSKA E JOHANNISSON T & LIPSKI J 1979 J Physiol 293, 173-197
JANKOWSKA E JOHANNISSON T & LIPSKI J 1978 Neuros i L tt Suppl 1 598

Recruitment f skeletal muscle capill ies vary the Permeabil ty-S face Ar Pro- Lct (PS) as an ndex f perfused micro- vascular exchange area
L.LIIE P PAASKE & PER SEJRSEN
Dept. of Int Med P Rigshospital t & Inst. Med Physi l B Univer ity of Copenhagen Denmark

As shown by Krogh (1919) the numbe f op- en muscl capillaries per unit cros ect ional area is larger during exe is than during rest. The degree f this recruit ment is controver ial I th pr sent stu dy recruitment was examined in the cat using the isol ted gastrocnemi pr para tion which provides ingl inl t ingl outlet autoperf sed and non ecircul ting system. PS (=CDC) was determined by the single injection method using esidue de- tection (indicators Cr EDTA & CO- BII) and venous ampling (C inuli n) The respons curves wer analysed as de- scribed earlier (Sejrsen 1970 1979 Paa ske & Nielsen 1976) If it i as umed that the average distance between perf sed c pillaries is 100 µm t t the aver ge intracapillary adiu i 3 2 µm and the average capillary length L i 1200 µm we can estimate 1) capillary surface area per g tissue S=2πrLN (cm²/g) 2) capillary open pore area pe g tissue S PSΔx_d/D (cm²/g) 3) numbe f perfused capillaries per g tissue N 4) inter pillary distance (cm) 5) volume f open apillaries per g tissue V=πr²LN (cm /g) Δx_d is the diffu- ion di tance acros th membran (1000 Å) and D i the f ee diffusion coeffi ient in wat r Experimentally it wa found that PS/D=CDC/D=53 82 3 27f (-0 87 p<0 01 f plasma flow i ml/100g min) ee Fig 1 wh e the es lts p esented The in di te that in k l tal mu l ecruit ment f capillari is lin function of pl sma flow When plasma flow incr ases from 1 to 50 ml/100g min ecruitment ta kes place with a fact f 4 o 4 time mor pill i ar perf ed during heavy ex cise than du i g t

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D 54

Rectified rectified smooth EMG d ing b iek m iron luntary us le nt cti

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Medical Department P Bi pebj g Hosp t l and Dan Nat As I f P l Hell p Denmark

It has been demonstrat d that the re han ism f brisk maximum l t y mus l contra tion (MVC) i b pha i notch ppe pp imat ly 337 ms afte th ontr tion i initi ted (Secher t l 1978 Rube & Se he 1979) The pres t tudy de cribe the rectified smooth EMG (REMG) t ppe when veraged ral cont a ti ns
Six healthy bj t mal and f mal with pre ous pe i no f om imil periment pe formed pe t d thumb p sure MVC once ry fifth se ond f four minutes Force wa regi t red on t i Entage dynamomet nd ord d on y t with na ural freque y f 293 H REMG was re rd d di tly (Fig 1 t p) or frequency modul t d nd tored on t pe recorder The 48 REMG was a reg d on Hewlett Pa k rd 1000 21MX comp t ith low pass filt t on llowing ise time of 107 msec
The notch i he me hanogr occured t 316 msec (Fig 1 top) The REMG howed large aplitud t the beginning f the sent ction f llowed by pl t t lower level When ve aged ov th 48 MVC the "noi y ppe re ce f the REMG disappe red (Fig 1 bottom) For the i subje ts th pe k REMG mplitud ppe d t 157 3 (E) ms c t h ed t 310 47 ms and th pl t had a- plit d f 63 8 3% f th pe k
The ult ugg st that th us l fi br i l ed i tabl hing th fi t pa t f th MVC me h gr h larg EMG p th the l fib re pon ibl f th re t f th t ti

Refere ce
SECHER N H RØPSGAARD S & SECHER O 1978 A t Phy i l S d 103 456-462
RUBE N & SECHER N H 1979 A ta Phy i l Scand uppl 473 34

F g 1 (t p) Me hanogr (f) d REMG f ngl t a t (Bottom) REMG ve aged 48 MVC

1 sec

D 51

Sympathetic regulation of adenosine in the rabbit heart

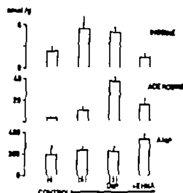
BERTIL B FREDHOLM LOUISE VERNET PER HEDQVIST+ and MARIANNE WENNMALM+

Departments of Pharmacology and Physiology+ Karolinska Institutet S-104 01 Stockholm Sweden

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D 52

Similariti between synaptic action of Ib tendo gan aff ents and Ia muscle pindl aff rent upon pin l mot neu on

By E JANKOWSKA R HACKEL & D McCREA

Department of Physiology Univ rsity of Göt borg Göteborg Swed n

Group I inhibition of homonymous and synergistic motoneurones and group I oligosynaptic xcitatio have been attributed to the action f Ib tendon organ aff rents Rec tly it has been shown (Fetz et al 1979) that group Ia muscle spindl aff rents can also nt ibut to th autogenetic inhibition of homonymous motoneurones The pr e t results tend this obs rvatio and show that I afferents ontri but both t the inhibition of synergists and to the oligosynaptic citation f motoneurones as w ll

Since small (< 40 μm) brief triangula str tch s of triceps su ae s lectively activat the Ia afferents (Brown t al 1967) this technique was used to evok synaptic ac tions of Ia afferents in the motoneuron s hich inn rvat the f llowing mu cl s of the c t hindlimb; po t biceps semitendinos s gracilis, semimembranosus-ant biceps quadri ceps tibial flexor digitoru longu , and pe oneus tertius and brevis Under chloral anaesthesia and muscle paralysis the triceps su ae was tonically stretched to an initial tension of 5 N, the brief stretches applied and intracellular ffects recorded from lumbar motoneurones A signal averager aided i the d tection of small amplitude postsynaptic ef fects The r s lts demonstrate that variety of oligosynaptic ffects result from activation of only the Ia muscle spindl afferents The segmental latencies of thes effects (1 1-4 ms) ar c mpatible ith di and trisynaptic link ages in thes pathways (Eccles et l 1957) IPSPs we e evoked i a large p oportion of the tens r motoneurones hil EPSP predominated in the flexo motoneurones This p ttern is similar to the one d scribed for the actions of Ib tendon o gan affere ts (Eccles t al , 1957). A numbe of motone ron s wer found which dis played both oligosynaptic IPSPs and EPSPs; a r ponse not d cribed for actions f the Ib fferent Th s esult together with previous observations on the conve gen of group Ia and Ib aff rents upo cert in interneuro s (Jankow k et al , 1978) suggest that common int r neuron s may be involved in mediating the ac tions of Ia and Ib aff rents to motoneurones

R f renc

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itative gamma-radiation autoradiography for
flow measurement

HULTBORN & L. WEISS

Departments of Histology Radi therapy Physiology Surgery University of Göteborg Sweden

... blood flow can be measured using several ... procedures ... drop recorders ... electro... and ultrasound flow meter ... plethysmo... and during the past two decades by di tri... of various diffusabl and non-diffusabl ... injected in the vascular system or the ... subject to tudy Using ... the micro... trace technique lumps of tissues of interest are dissected out and analysed for radioac... These lumps are rarely subject ... hi... ical tudy and often het rogeneities regarding ... phology and function are overlooked This i ... cially true in the tudy of tumour circula... where such heterogeneities generally ... are of theoretical and linical importance ... he microsphere trace technique was thus fur... developed to llow: 1 ... screening of per... ion heterogeneities 2 correl ion f perfu... with histology 3 quantitation f blood flow ... desired discrete regions of tissue

After injection of labelled spheres and drawing reference blood tissues f inter ... are dis... ... out and fixed in f rmalin Two ... thick ... tions are cut and placed upon an X-ray f lm ... ther plain or combined with intensifying ... The reference blood i heparinized and ... ially diluted In each dilution the spheres ... allowed to settle onto ... tandardized area of plas i film. Thi film is placed togethe with the issue sections on the X-ray film. Af er an exposure time f weeks the densi y f blackening from the tissue section can be compared with the ref rence blackening and blood flow at any point can be expres ed as volume blood/weigh f fixed tissue and ... Hi tologi l sections can be produced from the fixed autoradiographed ... tion ... provide morphological ... lation

Two different isotopes can lso be used and analyses i accomplished ... by radiation quali ty discrimination (... ^{125}I & ^{141}Ce) or by ... al discrimina ion (... ^{125}I & ^{99m}T)

The procedures thus llow identification of heterogeneous perfusion quantitation of blood flow in any desired region as well as morphological correlation to func ional data

Ref rence

HULTBORN R. & WEISS L. 1979 Microvascul Res 17 S120

D 58

...ular reacti ity ... experimental mammary ... and normal vascula beds ... studied i ... with rtifi i l perfusion

WEISS E TVEIT & R HULTBORN

Departments of Phys ology Surgery Histology and ... Radiotherapy University of Göteborg Sweden

... vivo studies with microsphere technique (We ss ... 1 79) on induced and autotransplanted DMBA ... methylbenzanthracene) rat mammary neoplasi ... demonstrated that the tumours have a rel ti... high resting blood flow and strong response ... noradrenaline indicating rel tively low ... resting tone in the tumour vascul r bed Skin to ... which the tumours re histogenetically rel ted ... under the same conditions low resting flow ... slight response to noradrenal ne indicat... high resting tone Due to the varying re... resting vascul tone the different or... in the i vivo situation it is difficult to ... evaluate the rel tive reactivity to pressor drugs ... Against thi background it is dvantageous to ... an i vitro art fi i l perfusion technique ... the hemodynami characteri tics can be ... subject to more detailed analysi from maximal ... vascular relaxation to maximal constriction Thus ... eviscerated rats with DMBA induced mammary neopl... were artifi i lly perfused and microspheres ... with three different isotopes were ... jected t ... different points of the pressure/flow and dose/ response curves for nfused noradrenaline tarting ... at maximal relaxat on By using the microspheres the flow di tribution rel tionships between the different organs could be tudied It was shown that the tumour ascul bed had pressure flow curve during maximal relaxation which we ... ituat ed between that of skin and that of muscl During stepwise noradrenal ne infusion the tumour vascular bed responded f more than any of the other vascular beds studied such a mus le ski liver salivary gland kidney spleen and uterus The organ which was losest i characteristics wa sk n

It wa concluded that the blood vessels supply ing the DMBA induced mammary tumours have resi tance during maximal relaxation withi the range of such vasculs beds as muscl and ski and that they are extremely reactive to noradrenaline even as compared to thei histogenetical origin skin Whether this increased reactivity to noradrenaline i due to the lack of nerve endings remains to be investigated

Reference

WEISS, L HULTBORN R. & TVEIT E 1979 Microvascular Res 17 S119

D 55

The effect of salmon calcitonin (SCT) on the total plasma calcium in a marine teleost fish

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Calcitonin (CT) is known to be produced in the *ultimobranchial glands* and present in relatively high concentrations in the blood of both bony fish and elasmobranchs

Although fish calcitonins show very high potency in producing *hypocalcemia in mammals* experiments on fish have mostly given negative findings Thus salmon calcitonin (SCT) does not affect the calcemia of salmonids (Pang 1971 Copp et al 1972 Milhaud et al 1977) and neither has eel calcitonin affected the calcemia of the Japanese eel (Yamauchi et al 1978)

A series of experiments have been carried out to test the effect of SCT (SANDOZ) on the calcemia in *the Atlantic cod Gadus morhua L* Doses of 5 8 10^{-9} 1 0 10^{-8} mol/kg were used and injected i m or i a at t 0 Group size varied from 5-8 and control groups received corresponding amount of the SCT solvent

Exp I and II were carried out on fish in normal sea-water ([Ca] 10 mM) Exp I tested for any short term effect on calcemia (blood samples taken at t -60 0 10 30 60 120 240 min) and exp II tested for any long-term effect (blood samples taken at t=0 6 24 hrs)

Exp III and IV were carried out on fish placed in *hypercalcemic sea-water ([Ca] 100 and 150 mM respectively) at t=0 and blood samples taken* at 2 hr intervals from t=0-10 hrs and at t 24 hrs

Exp IIIb was identical to exp III except the SCT dose was given at t 6hrs instead of t0

No change in calcemia was noted in exp I and II *In exp III and IIIb* both the experimental and control groups developed hypercalcemia (from about 2 5 mM up to 3-4 mM) within 2 hrs which persisted throughout the exp No effects of SCT were noted in either exp *In exp IV the calcemia increased* steadily in the control group till death occurred whereas in the experimental group the increase halted at t 6hrs and no further increase occurred between t 6-10 hrs All fish in both groups died within t=24 hrs

The following can be concluded: a) CT does take part in preventing hypercalcemia and its endogenous production may become a limiting factor under extreme conditions *b) The endogenous control* of the calcemia is very strict as CT is unable to affect it under stable conditions (normo- or hypercalcemic)

Experiments designed to study the effect of a long-term SCT treatment of the cod and the reactions of these fish to hypercalcemic environment are now in progress in this laboratory

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D 56

Inhibition of epoxide hydrase by heavy- and organometals in vitro

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Turku Finland

The effects of Cd Hg^{++} Cu^{++} Zn Ni^{++} Co^{++} Cr^{++} and Ag as well as methylmercury dimethyl cadmium and p-chloromercurobenzoate were studied on rat hepatic microsomal epoxide hydrase (EH) (styrene oxide SO benzo(a)pyrene-4 5-oxide BPO) in vitro

1 The effect of buffer and pH used was critical Cadmium was strongly inhibitory only in tris buffer pH 9 but not in phosphate buffer pH 7 or carbonate buffer pH 9 Inhibition by Zn and Ni was seen in carbonate and tris buffers pH 9 but not in phosphate buffer pH 7 The inhibition by Hg and Cu was stronger in phosphate and carbonate buffers than in tris Co and Cr were poor inhibitors in the conditions tested Ag was strong inhibitor of EH (as well as monooxygenases but poor inhibitor of glutathione S-transferase)

2 Some of the metals were scavanged strongly from the medium by microsomal membranes This was concluded from the facts that a) Dilution of the enzyme preparation (microsomes) increased the inhibitory potency b) Hydration of BPO (which as a sensitive method needs only one 40th part of the enzyme amount needed in measurement of SO hydration was much more sensitive to these metals than hydration of SO (a common enzyme is involved) Especially sensitive to the amount of microsomal lipid present in the incubation were Ag Hg and Cd

3 Methylmercury and dimethylcadmium were also inhibitory to EH The inhibition was much more pronounced in tris (pH 9) than in phosphate buffer (pH 7) p-Chloromercurobenzoate inhibited the hydration of BPO potently in phosphate buffer (pH 7 but only slightly in tris buffer (pH 9) Strong binding to microsomal lipid was likewise evident

The present data indicate that many heavy metals and organometals are inhibitors of epoxide hydration Detection of the inhibitory effect is strictly bound to the conditions used

Grants NIH R01 01684 Juho Vainio Foundation Finland

...ibitory connections from different types of principal ...ls to perigeniculate neurones.

...AHLSÉN, G. LINDSTRÖM & F.-S. LO

...pt. of Physiology University of Göteborg, Sweden

...incipal cells in the lateral geniculate nucleus receive ...ibition through a subcortical recurrent pathway ...ich involves axon collaterals of principal cells and ...ibitory interneurones in the perigeniculate nucleus ...e convergence of excitation from different types of ...incipal cells onto perigeniculate neurones has now ...en investigated in acute experiments on pretrigeminal ...ts.

Two types of perigeniculate cells were found. One ...pe was excited from sustained, slowly conducting princi...pal cells; the other from transient, rapidly conducting. ...th types received convergent connections from "on-...ntre" and "off-centre" principal cells. Their recep...ve fields were round and about twice the size of the ...ld centre for the corresponding type of principal cells.

In contrast to the principal cells the majority of th ...rigeniculate neurones wer binocular indicating that ...ey receive excitation from principal cells in both la...ers A and A_1 of the lateral geniculate nucleus. The ...rigeniculate cells in turn had axonal branches which ...inate in both laminae.

These findings demonstrate that visual signals from ...e two eyes and from both the "on-centre" and the "off ...ntre" systems are mixed at the interneuronal level in ...e recurrent inhibitory pathway to principal cells.

...alcium sensitivity of isolated mesenteric resistance vessels in spontaneously hypertensive rats

M.J. MULVANY, J. CHRISTENSEN, K. NYBORG

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Studies on isolated arteries (Hermsmeyer 1976) and resistance vessels (Mulvany et al. 1979) show that if neuronal uptake is inhibited the noradrenaline (NA) sensitivity of SHR vessels is greater than that of WKY vessels. In the present study we have investigated possible causes of the increased NA-sensitivity.

Small mesenteric arteries (internal diameter ~150 um) from 3-4 month old SHRs and WKY were mounted as described previously (Mulvany & Halpern 1976) but here on double myograph permitting paired experiments. Vessels were depleted of Ca^{++} by repeated stimulation in Ca-free solution (containing 0.1 mM EGTA). They were then exposed successively to solutions containing 0.05, 0.1, 0.2, 0.4, 0.8, 1.6 and 2.5 mM Ca^{++}. All solutions also contained 3 µM cocaine to prevent neuronal uptake. They were held in each solution for a period of 4 min being maximally activated during the second half of each period by the addition of 10 µM NA.

In each of 5 paired experiments the SHR vessel responded to lower Ca-concentration ($Ca\ ED_{50}$ 0.10 ± 0.02 mM (SE)) than the WKY vessel ($Ca\text{-}ED_{50}$ 0.18 ± 0.02 mM). Corresponding experiments with K-stimulation (with 1 µM phentolamine for α-receptor blockade) showed by contrast little difference between SHR and WKY vessels either in their K-sensitivity or in their Ca-sensitivity in respect to K-stimulation.

The results suggest that the greater NA sensitivity of the SHR vessels compared with the WKY vessels is due not so much to differences in the calcium permeability of their plasma membranes but rather to a specific difference in the effectiveness of their adrenergic receptors in utilizing extra- and intracellular calcium.

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D 59

Emotional stres increase plasma adrenaline and cyclic AMP in unrestrained rats

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Plasma cyclic AMP and techolamines were determin ed on samples withdrawn from unrestrained rat via chronically implanted aortic catheters The rats were trained to acquire a conditioned avoidance reflex i two-way shuttle box The unconditioned stimulus was an electric shock (0 5 1 mA) d livered every 1 r 5 mi via metal rods forming the floor of the box The conditioned stimulus consist ed of ound and light signal deli ered 5 s before the lect i shock

Initial experiment (rats conditioned with 1 mA shocks every 5 min) showed ignifi ant in reas in plasma cyclic AMP from 12 1 1 2 to 18 3 3 4 nM (n=7 p<0 05) after 30 min stre with no change i controls In second set f expt (rat condi tioned with 0 5 mA every min) plasma cycli AMP increased less Fig 1 hows the rel tionship between arterial plasma techolamines and cyclic AMP in these animals It an be seen that low resting levels of t cholamines prevailed and th t tre increased adrenalin but not noradrenalin l vels There was correl tion between level f cyclic AMP and adrenaline but not noradrenaline in plasma Our results suggest that adrenaline may be f majo importance for the increas i plasma cycli AMP een i rat during tres Thi i in agreement with bservations that adrenalin i con iderably more pote t timulator f plasma cycli AMP than i noradrenaline Expts with β-blocking drugs progress to furth d fin the rol of adrenaline the cycli AMP response t stress Work i also sc duled t study the effects of diff rent kinds and tensities f stress on the *secretory response fr* sympathetic nerves and adrenal medulla

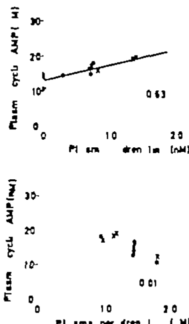


Fig. 1: Relationship between art rial plasma catecholami es and cycli AMP i rats during (x) and before or aft ( ) conditioned voidance stres

D 60

A Comparison of Skeletal Muscle Morphology, Meta bolism and Function in Smokers and Non smokers

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The impaired physical performance capacity in to bacco smokers *is probably partly related to the* effects of smoking on the muscle tissue itself This seems conceivable since physical working capa city and metabolic capacity of the working muscles are closely interrelated (Holloszy & Booth 1976) It was therefore thought to be of interest to compare a group of smokers and non-smokers from a homogenous population of men with respect to skele tal muscle morphology metabolism and function This population had earlie been used to study the effects of age on these variables (Larsson 1978)

Fourty three healthy males (18 smokers and 25 non smokers) all white-collar workers volunteered for the study Samples were taken from the vastus lateralis muscle for histochemistry enzyme assays and electron microscopy using the needle biopsy technique The muscle fibres were stained for myofibrillar ATPase and NDAH activity The muscle fibres could then be classified into typ I IIA IIB and IIC fibres and their fibre area determined Activities of muscle enzymes representing key enzymes of skeletal muscle energy metabolism were *measured* (PFK LDH CS CYTOX and HAD) Stereo logical analyses of the *mitochondries were perform*ed on electron microscope micrographs In the knee extensor muscles the maximum isometric and dynamic strengths were measured at velocities corresponding to 0^0 60^0 120^0 and 180^0 x s $^{-1}$ using an isokinetic dynamometer

The proportion of type I fibres was lower (p< 0 001) in smokers compared to non smokers (38 1± 2 3%; 50 7 2 5%) Type II fibre subclassification revealed a correspondingly increased incidence (p<0 01) of type IIB fibres in the smokers Fibre areas did not differ in the two groups Muscle oxidative capacity was lowered in the smokers as judged from decreased (p<0 05-0 001) mitochondrial enzyme activities and a lowered (p<0 01) fibrillar space mitochondrial volume fraction Isometric and dynamic strengths were lower (p<0 05 0 01) in the smokers except at the highest velocity of movement studied

The present result show some clear-cut difference in skeletal muscle morphology metabolism and func tion between smokers and non smokers The reduction in muscle oxidative capacity and strength in the smokers may at least partly be explained by the *difference in fibre type* distribution These differences may either be explained as an effect of smoking per se (e g the stimulating effect of nico tine on the muscle fibres at the motor end plate and its interference with acetylcholine metabolism (Fische et al 1960)) or it may be that background factors are responsible such as genetic differences

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...t of the vasoactive intestinal polypeptide ... the intraocular pressure (IOP) and regional ... flow

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...facial stimulation of the facial nerve causes ...dilation in the choroid and rise in IOP ... effects seem to be mediated by non-cholin... fibres (Bjernschantz & Bill unpublished ... Histochemical studies by Uddman et al ... communication) have indicated that ... VIP-containing neurons in the facial ... VIP was discovered and named on the ba... of its vascular effects (Said & Mutt 1970) ... physiological role i not known (Said 1975) ... have investigated the effects of intra... as well as intravenous injections of VIP on ... and the regional blood flow measured ... labelled microspheres Intravenous as well ... intraarterial injections in rabbits caused ... in IOP at dose not affecting the blood pre... a dose of 500 ng/kg b w given over ... intravenously there was little chang i ... arterial blood pressur but marked vasodila... in several tissue Flow increments in the ... gland and pancreas were more than 100 % ... 100 % in heart muscle and 25 50 % in the ..., choroid plexus thyroid and sub-mandi... gland No consistent vasodilation was ob... at this dosage in the ... liver adrenals skeletal muscle and skin Injection f VIP into th anterior chamber caused marked vasodil ion in the anterior uvea

The esults indicate that VIP at low doses produces selectiv changes in the distribution of the cardiac output, which may be of therapeutic interest The effects in the eye suggest that if VIP is liberated in the eye during facial nerve stimulation it may well be responsibl for the vasodilatio caused by the stimulatio

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D 63

E haustion in rei deer calves

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Reindeer management at times specially i the coral implies a high train on the animals and certain loss of animals may occur Optimal weather conditions may reduce this risk It h s b en sp c lated on why rei deer an animal with highly developed running skills sometimes l y down for one or several reasons d nying to stand on its feet Some of th se nimals will finally die Hyperthermic collapse has been u ed an xplanation for at le st some of these cas of death

Having had acces to hardworking rein deer during taggi g of calves in J ly rectal temperatures of faste ed calves w re meas red 7 10 cm i to the rectum by means f a thermistor which wa calibrated agai st a precision th rmomet r The anim ls re running i a coral during tagging O ly th cal s may obtain some food in the pen In thi st dy I have co ntrated on th calves the high risk group concerning collapse from xha stion

R ctal temperat res of 20 mal and 30 female normal c lves registr ted at i terv ls of a five h ur tagging period w s 40 7 0 7(SD) °C and 40 6 0 5(SD) °C respecti ely A tre d of decre se i t mperat re with time i the pen was s on in the data Irrespecti e of e mea temperature of the first 15 registration 41 1 0 4(SD) °C was compared with the mean of the 15 last registrations 40 2 0 6(SD) °C The decrease in mean temperature of 0 9°C was sig ific t p <0 001 as tested by Student s t test

Fin lly rectal temperature of 13 collapsed calves was 39 3 0 4(SD) °C highly significantly below the mean valu of all the more normal groups refered to above p <0 001 when compared with the l last registrated (Student s t test) Those 13 c lves showed the typical *symptoms of e haustion and data from th* are ot included in the more normal grou refered to abo e

In o e of th cal s which died from hau tio dissection for fat stores revealed a complete lack of fat in the axillar inguinal nd ren l regions areas where some fat is s ally f und

These data indicat that hyperthermia is not the main ca s of mortality in these e ha sted anim ls as they ha e lower core temperat re than controls E haustion how ver is not yet a well defined co ditio in any mamm l The problems of limiting factors in e ha sti ercise are far from sol ed Depleted energy res rves obvio sly is factor of import nce When looking for th causes of d ath in e ha sted rei deer congesti f heart l ng edema and shock sho ld of co rse be taken into consideration in ddition t depleti of a ail ble nerg

D 64

The blocking effect of diphenylhydantoin on par hyd oxymerc riben oate treated rat ph enic nerve di phragm preparation

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Pretreatment with 0 1 mM parahyroxymercu riben oate (pOHMB) increa ed the blocking eff ct of diphenylhydantoin (DPH) (0 5 1 0 mM) on the indirectly stimulated(0 1 Hz) rat diaphragm The present periments were performed to describe and localize this effect

pOHMB is a SH-group inhibitor and the increased block was dependent upon the reaction between pOHMB and SH groups since SH-group protection with dithiothr eitol (Cleland 1964) abolished th combi ned effect of pOHMB and DPH

The muscle contracted upon direct stimulation after the contraction to indirect stimulation had ceased Tests on the iso lated phrenic nerve did not reve l n i creased blocking effect of the combina tion of pOHMB and DPH This indicated th t the block should be localized to th neuromuscular transmission proces

A tenfold increase in the Ca^{++} concen tration slightly increased the block Thi indicated that the block was not du to competition with the Ca^{+} flux into the nerve terminal leading to decr ase of acetylchline release Decreased presynaptic excitability or decreased postsynaptic receptor sensitivity may explain the Ca^{++} potentiation of the block

In order to separate between a pre- or postsynaptic mechanism of action micro-electrod technique with recording of mi niature endplate potentials (MEPPs) and endpl te potentials (EPPs) was used MEP could be recorded after contraction had ceased indicating that the block was du neither to acetylcholin depletion nor to inhibition of the receptor sensitivity EPP were r corded in the cur rized pre par tion where the action potentials were abolished The EPPs disappeared abruptly after a period of slight decre s of amp litude fter DPH additio

A significant time diffe ence to cease of endplate pote tials between the pOHMB treated pr paration and the preparation wher DPH was added lone ugg ted that an inte fe ence with th excitability of the nerve t rminal w the u e of th block

Refer nce

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